What Makes A Ferox? The Drivers & Consequences Of Alternative Life History Strategies In *S. trutta*

Martin Robert Hughes

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Scottish Centre For Ecology And The Natural Environment,
Institute Of Biodiversity, Animal Health And Comparative Medicine,
College Of Medical And Life Sciences,
University Of Glasgow

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ABSTRACT

Understanding the mechanisms involved in producing and maintaining variation in phenotype, behaviour and life history traits within a single species, is of fundamental importance to evolutionary biology. Such intraspecific variation is well observed in organisms inhabiting depauperate environments, exploiting uncontested trophic niches. Classic examples can be found in island communities such as Darwin's Finches of the Galapagos Islands and the *Anolis* lizards of the Caribbean, where organisms derived from a single invading ancestor rapidly diversified into numerous ecologically distinct niches, forming distinct and often reproductively isolated populations. Such adaptive radiations are considered an essential prerequisite to species formation and is often referred to as ecological speciation.

Freshwater lakes share characteristics similar to islands that enable ecological speciation to occur rapidly. The rapid proliferation of African cichlids species in the African Great lakes is perhaps the best example of ecological speciation found on Earth today. Thousands of species of African cichlids have been described within Lakes Malawi, Tanganyika and Victoria each adapted, often morphologically, to a particular ecological niche. Fish species inhabiting post-glacial lakes of the Northern hemisphere represent model organisms to study the early mechanism of ecological speciation. Species such as Arctic charr and Three-spine sticklebacks have diverged across ecological axes within a single lake in numerous systems. As post-glacial lakes were inaccessible to ancestral fish until the last glacial retreat, (approximately > 15 000 years ago) the observed divergences have occurred in an extremely short amount of time.

Brown trout *Salmo trutta* can exhibit multiple life histories within a single lacustrine system, including benthic, pelagic and piscivorous forms. The piscivorous form, known colloquially as ferox trout, are a relatively rare, understudied form of the *S. trutta* species complex. Interestingly ferox trout not only exhibit discrete morphology and foraging behaviour they also have higher growth potential, delayed maturation and extended longevity compared with sympatric *S. trutta*. Indeed, ferox trout represent a genetically distinct ancestral lineage in some locations where they occur, however the ecological, physiological and behavioural mechanisms driving the production of ferox trout populations has yet to be investigated. Thus, the thesis presented here tests the importance of different drivers in ferox trout production to increase our understanding of species formation and ecological speciation. Furthermore, I also investigated the within lake movements (home range, core range, speed) of benthic and pelagic *S. trutta* occurring in sympatry to determine

differential habitat use. In the General Introduction (chapter 1) I extensively review the available literature on adaptive radiations and ecological speciation with a particular emphasis on fish in post-glacial lakes. I discuss the history of ferox trout to contextualise the current thinking and to highlight knowledge gaps within the research.

To investigate the ecological drivers of ferox trout populations, fine scale environmental characteristics associated with known ferox trout populations were investigated. I found large, deep lakes with populations of *S. alpinus* were highly correlated with ferox trout populations. I found 192 lakes in Scotland have evidence of supporting ferox trout and 366 lakes in Scotland could theoretically support ferox trout based on lake area alone (chapter 2).

The alternative growth strategies and life spans exhibited by ferox trout and sympatric benthivorous brown trout (benthivorous trout hereafter), were examined by comparing the growth trajectories and age structures of three sympatric populations (Loch Rannoch, Loch Awe and Loch na Sealga). In Loch Rannoch, the ferox population adhered to the conventional model of growth proposed for ferox trout, i.e. relatively slow growth followed by an acceleration of growth after a switch to piscivory, however the two other populations did not conform to this model of growth. In Loch Awe, ferox trout grew much faster than benthivorous trout, including early ontogeny, however in Loch na Sealga there were no measurable differences in growth. Interestingly, all ferox trout populations were older than benthivorous trout. These results demonstrate that there are multiple ontogenetic growth pathways to achieving piscivory in *S. trutta* and that the adoption of a piscivorous diet may be a major factor contributing to extension of life span in *S. trutta* (chapter 3).

Physiological, morphological and behavioural drivers were investigated by rearing full-sibling families of sympatric ferox trout and benthivorous brown trout from eggs in the laboratory, under common garden conditions. I found offspring from ferox trout parents had higher survival rates, larger yolk sacs and decreased levels of mesenteric fat, compared with offspring from sympatric benthivorous trout. Offspring of ferox trout and benthivorous trout also had distinct head shape morphology, which converged over time under common garden conditions (chapter 4). Offspring from ferox trout were more dominant than size-matched offspring from sympatric benthivorous trout, by examining food acquisition ability, spatial position, flank colour index and aggressive interactions within a semi-natural stream system (chapter 5).

Lastly, the within lake movement of sympatric benthic and pelagic *S. trutta* was investigated by acoustic telemetry. Acoustic tags were surgically implanted into *S. trutta* exhibiting distinct head morphologies associated with trophic position in an oligotrophic

post-glacial lake. Tagged *S. trutta* were tracked over a three-month period (July-September). No differences in home range or core area size (km^2) between benthic and pelagic *S. trutta* were identified. However, both forms demonstrated clear diel cycles in movement and responded similarly to temporal change. There was extensive overlap in core use of the lake, however pelagic trout were found over deeper waters more often than benthic trout (chapter 6).

In the general discussion (chapter 7), I summarise the results of these studies and discuss the evolutionary, conservation and economic importance of such research. I also discuss the limitations of this research and the potential future areas of study.

“It seems to me that the natural world is the greatest source of excitement; the greatest source of visual beauty; the greatest source of intellectual interest. It is the greatest source of so much in life that makes life worth living.”

SIR DAVID ATTENBOROUGH

“When you want to succeed as bad as you want to breathe, then you will be successful.”

ERIC THOMAS

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DECLARATION

I hereby declare that the material presented in this thesis is the result of original research, conducted between February 2013 and January 2017, under the supervision of Professor Colin E. Adams. This thesis has not been submitted, in whole or in part towards the fulfilment of any other degree. It is entirely my own composition and that the research described herein was carried out by me unless otherwise stated or acknowledged.

Signature _____

MARTIN HUGHES
JANUARY 2017

CHAPTER 1.
GENERAL INTRODUCTION

1.1. HISTORY OF EVOLUTIONARY THEORY

Since the publication of Darwin's eminent work "On the Origin of species" in 1859 and his proposal of natural selection, evolutionary biologists have investigated and tested the theory ever since. The field of evolutionary biology has itself evolved into a multi-faceted discipline that incorporates cutting edge techniques at the forefront of science, where genetics and ecology are often used in conjunction to develop an understanding of this fundamental question: How do species originate, and what drives the formation of species we see today? An overview of all the proposed theories and works fall out-with the scope of this research, however it is important to introduce the timeline of evolutionary theory to contextualise the way in which we approach evolutionary biology today.

The amalgamation of Charles Darwin (1809-1882) and Alfred Russel Wallace's (1823-1913) concept of natural selection was published around the mid-19th century resulting in a concept we now call 'Darwinism'. Darwin's definition of natural selection is still used today - "One general law, leading to the advancement of all organic beings, namely, multiply, vary, let the strongest live and the weakest die." Darwinism made five fundamental assertions: (1) Organisms reproduce with one another; (2) more offspring are produced than survive to reproductive age; (3) variable traits are present in all individuals of a population; (4) individuals with the best suited traits to the environment are more likely to survive (Darwin) or endure (Wallace) than individuals with less suited traits; (5) these traits are heritable and passed down to the next generation, or accumulate within a population. A major issue for Darwin at the time was explaining the final assertion: how are traits heritable? The popularisation of Darwinism by Wallace (1889) was developed further by August Weisman (1834-1914) in 1892 who proposed sexual selection creates new, variable populations of individuals on which natural selection can act upon and therefore determine evolutionary change on the best suited variables. This expansion of Darwins theory was coined neo-Darwinism by Romanes (1895) and persisted until the early 1900's.

It wasn't until the posthumous rediscovery of Gregor Mendel's (1822-1884) work on inheritance in 1900 that the concept of genes was first considered, initiating the field of Mendelian Genetics. In 1918 Ronald Fisher was the first to validate Darwin's theory through Mendelian Genetics with his publication 'The Genetical Theory of Natural Selection'. Fisher outlined many important concepts which subsequently led to the publication of other

important texts (Dobzhansky, 1937; Mayr, 1942; Rensch, 1947; Stebbins, 1950; Simpson 1944, 1953); which helped define what is now known as the modern evolutionary synthesis.

Thereafter the modern synthesis focused heavily on the genetic mechanisms underlying evolution and improvements in molecular biology techniques progressed and strengthened the theory of evolution substantially (West-Eberhard 2003). The role of the organism itself, and its phenotypic plasticity was largely dismissed during this time however, and considered 'noise' (Pigliucci *et al*., 2006; Noble, 2011). More recently, the importance of an organism's environment in shaping evolution and speciation has been investigated. Phenotypic plasticity is often defined as the ability of a single genotype to exhibit multiple phenotypes when exposed to different environmental conditions.

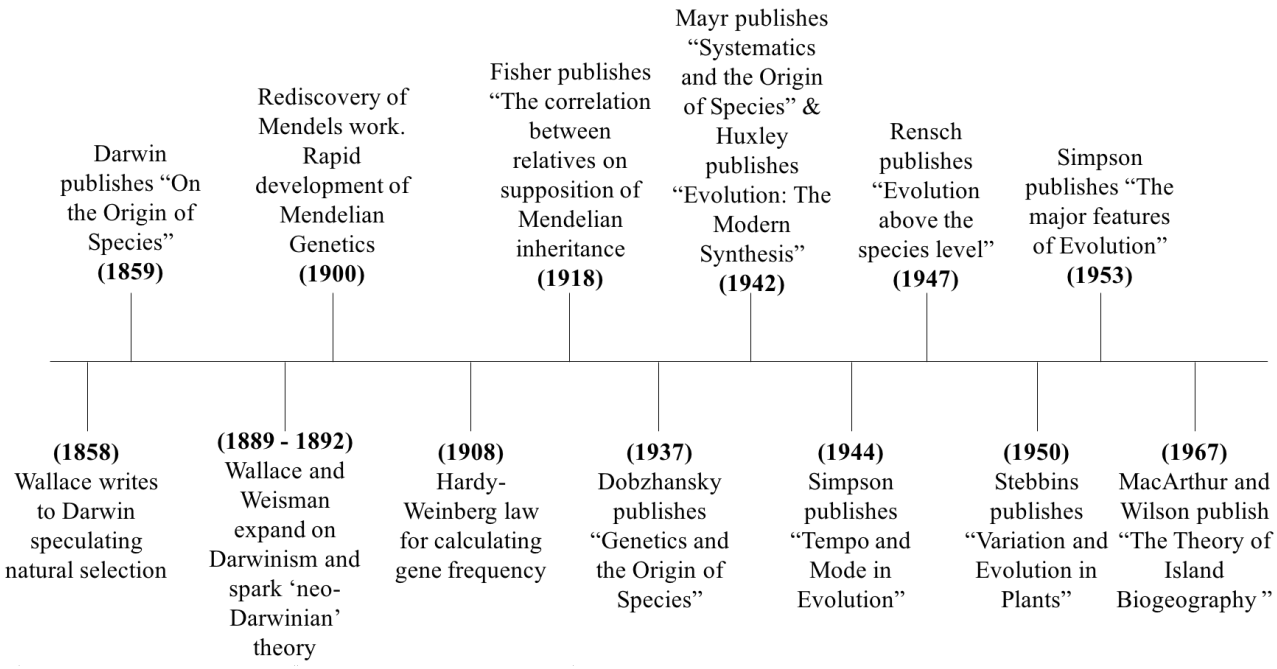


Fig. 1.1. Chronological timeline of evolutionary theory

1.2. Adaptive Radiation

Adaptive radiation can be defined as rapidly multiplying lineages exhibiting diverse phenotypes and inhabiting distinct niches within an ecosystem, derived from a single ancestral species (Simpson, 1953; Schluter, 2000). Adaptive radiation can be characterised by four fundamental criteria: (1). Adaptive radiations occur from a single common ancestor; (2) There is a clear relationship between phenotype and environment in radiating taxa, usually but not always exhibited by a clear morphological adaptation to the environment; (3) Such traits have a clear beneficial function to the organism, sometimes referred to as trait utility (Schluter, 2000); (4) The final criteria is rapid speciation, the emergence of multiple

diverse lineages in a relatively short space of time. Such bursts of radiating lines are characteristic of most examples of adaptive radiations (Simpson, 1953; Schluter, 2000; Seehausen, 2006). Here, we consider speciation, the formation of new species as the establishment of reproductive barriers between two populations, which may interbreed but produce inviable or infertile offspring; essentially Mayrs biological species concept (Mayr, 1942) with additional allowance of hybrids, as many sexually reproducing distinct species may hybridize without jeopardising the genetic integrity of discrete populations (Seehausen, 2004).

1.3. Ecological Opportunity

Schluter (2000) suggests 'Ecological Opportunity' is an important prerequisite required to prompt adaptive radiation within a taxon. Ecological opportunity (EO) is defined as 'a wealth of evolutionary accessible resources little used by competing taxa'- in other words, the availability of new unexploited niches (Schluter, 2000). Understanding how EO may arise is therefore extremely important in predicting and investigating adaptive radiations. Simpson (1953) was first to suggest four scenarios which may result in EO arising. First is the appearance of new resources which previously did not exist; (2) mass extinction of an ecologically dominant species; (3) evolution of a key innovation; (4) colonisation of depauperate environments. The logic often presented is that if ecological opportunity exists, organisms may diverge and produce ecologically distinct species, and thus ecological speciation can arise.

1.3.1. New Resources

The diversification of one clade may provide an ecological opportunity for another unrelated clade. Examples often used to describe such EO include the diversification of a plant species which in turn can be exploited by a herbivorous species (McKenna *et al*., 2009; Schuettpelz & Pryer, 2009). An example suggested by Simpson (1953) proposed the emergence of grasses allowed ancestral horse species to exploit this new resource and thus evolve themselves (Losos, 2010). A similar well-evidenced example includes the co-evolution of flowering plants (angiosperms) and weevils (McKenna *et al*., 2009). Through examination of fossil and molecular data, it's believed the current diversity of herbivorous weevil beetles (approx. 62,000 species) arose simultaneously with diversifying angiosperms throughout the mid-Cretaceous (McKenna *et al*., 2009).

1.3.2. Mass Extinction

The removal of ecologically dominant species may enable surviving species to exploit ecological resources previously unavailable to them. This type of EO is famously evidenced by the rise of diversity in mammals and birds after the Cretaceous – Tertiary (K/T) event which resulted in the mass extinction of non-avian dinosaurs. The removal of dinosaurs allowed mammals to diversify into new ecological niches, contributing to a complete re-organisation of ecological communities (Wolfe & Upchurch, 1987; Smith *et al*., 2010; Myers & Burbrink, 2012).

1.3.3. Evolution Of Novel Traits

The evolution of a new trait which enables an organism to interact with its environment in a novel way, without a change to the external environmental itself, is believed to be a mechanism through which ecological opportunity can arise. The evolution of such traits are referred to as 'key innovations'. Examples of key innovations include the evolution of wings in birds, bats and pterosaurs (Norberg, 1994; Bell *et al*., 2011), herbivory in insects (Wheat *et al*., 2007), adhesive toe pads in geckos and anoles (Warheit *et al*., 1999; Gamble *et al*., 2012) and the structure of nectar spurs in angiosperms (Johnson & Edwards, 2000; Sargent, 2004). An extremely interesting example of a key innovation is parity mode in squamate reptiles (Lynch, 2009; Pyron & Burbrink, 2014), particularly within the Viper group *Viperidae* spp. Oviparous and viviparous groups of vipers exist naturally in the wild, however the distribution of viviparous vipers appears to be correlated with cooler climates. Studies suggest the presence and evolution of viviparous reptiles in colder environments is common (Shine, 1983). Indeed, Lynch (2009) demonstrated through phylogenetic studies that the diversification of viviparous vipers remained consistent throughout colder periods of history (during the cooler Oligoene epoch), while at the same time oviparous groups declined. To this day in the northern hemisphere the most northerly distributed viper, found up to 69^{o}N, is a viviparous viper *Vipera berus*, the adder.

1.3.4. Colonising New Habitat

Finally, invasion of a novel depauperate habitat is believed to provide strong EO. Invasion of new habitats such as isolated islands may provide ecological opportunities to exploit resources previously not used by the invading taxa. The absence of predators may also enable taxa to use resources they are initially not well-adapted to and therefore result in niche shifts; compared with more diverse/competitive mainland ecosystems. Classic examples of ecological opportunity arising from invasion include the Galapagos finches and the anoles lizards on the Greater Antilles. Other examples include invasion of novel habitats

created by uplift of mountain ranges (Hughes & Eastwood, 2006) and exposure of habitats following de-glaciation (Ólafsdóttir *et al*., 2007).

1.4. Phenotypic Plasticity

An important factor which has historically been overlooked in adaptive radiation and ecological speciation is phenotypic plasticity - a species ability to exhibit multiple phenotypes from a single genotype (West-Eberhard, 1989). The ability to adapt to different niches from a single gene pool is likely a prerequisite for adaptive radiation to occur, as at the very least it enables the persistence of a species in a novel habitat (Agrawal *et al*., 2001). If phenotypes are 'plastic' then exposure to a new environment can result in previously unseen (novel) phenotypes at a much faster rate than genetic mutation or recombination can achieve. This rapid expression of new phenotypes can then be subject to selection and selected 'for' or 'against'. Thus, new characteristics enter the evolving population quicker and are subject to selection over a short time. This does not result in genepool change unless the trait under selection is associated with a particular genetic group in the gene pool, or expression of these new phenotypes are related to assortative mating, for example through differential habitat use, temporal and spatial place of breeding. Such adaptive plasticity allows organisms to maximise fitness within an environment (Stearns & Koella, 1986). Levels of plasticity between species is not equal however, some species are considered more plastic than others (Davidson *et a*l., 2011). An example of this would be the Galapagos mockingbirds *Nesomimus* spp, which have inhabited the Galapagos islands for the same amount of time as the Galapagos finches *Dendroica*, *Geospiza*, *Platyspize*, *Camarhynchus*, *Cactrospize* and *Certhidea* spp but have failed to diversify to the same extent as the finches (Grant, 1984). It is therefore assumed the finches have comparatively higher levels of phenotypic plasticity which allowed them to seize the ecological opportunity presented; resulting in the rapid proliferation of adaptive phenotypes we see today, whereas Galapagos mockingbirds have not. High levels of plasticity are therefore considered important to adaptive radiation. Polymorphic populations of birds, fish and insects which display multiple phenotypes within a population exist globally (Skúlason & Smith, 1995). Remarkably, some morphs are also characterised by life history traits and not morphology alone (Skúlason & Smith, 1995). Here I refer to life history traits as characteristics integral to an organism development such as growth rate, age at maturity and longevity etc.

Traditionally, the evolution of life history traits has been considered 'fixed' within a population. The field of life history evolution was first popularised in the 1960's and 70's by MacArthur and colleagues (MacArthur & Wilson, 1967; Murphy, 1968; Cairns *et al*.,

1969; Pianka, 1970;' Stearns, 1977) after their proposal of the r- and K-selection theory. MacArthur and Wilson (1967) proposed selection of a population's life history traits were influenced by resource-limiting environments and density dependant factors. They postulated "r selected" organisms were selected for, in variable and/or unpredictable environments that favoured rapid expansion of a population in a short amount of time. Alternatively, "K selection" favours organisms in more stable environments with high levels of intraspecific competition, where populations remain close to their carrying capacity. Although important for generalisations about life history traits, this theory fails to account for the 'within population plasticity' of life history traits.

I consider the most famous examples of adaptive radiations before discussing the importance of emerging studies and the current research of highly plastic species inhabiting novel depauperate environments.

1.5. Trophic Niche Specialisation

Lack's 1947 publication 'Darwin's Finches' is arguably one of the most important books on evolutionary ecology which served, and continues to serve, as the basis of study on the famous Galapagos Finches (Lack, 1947). Surprisingly, at the time of publication of the first edition, Lack believed the radiations observed in the finches were non-adaptive; a view which he later changed. Indeed, Darwin's Finches have since been used as the most famous example of adaptive radiation and adhere to the four principal criteria outlined by Simpson (1953) and Schluter (2000). Despite their significance to evolutionary biology, the phylogenetic relationship and verification of a common ancestor was not confirmed until relatively recently (Burns *et al*., 2002). Primarily, diversity among Darwin's Finches relates to their bill shape (Grant & Grant, 1996), although diversity in plumage and song have also been described (Ryan *et al*., 1994). Fourteen closely related species have been described on the Galapagos each with a bill shape reflective of the resources they exploit (Lack, 1947; Grant, 1981; Grant & Schluter, 1984; Schluter & Grant, 1984). Although subject to change, the current taxonomic classification of Darwin's Finches falls into four categories: the ground finches *Geospiza*; the tree finches *Camarhynhus*; the warbler finches *Certhidea* and the vegetarian finches *Platyspiza*. Within the six species of ground finches, three specialise on large, medium and small seeds respectively - the large ground finch *G. magnirostris*, the medium ground finch *G. fortis* and the small ground finch *G. fuliginosa*. Two ground finch species specialise on cactus flowers - the large cactus ground finch *G. conirostris* and the common cactus finch *G. scandens*. One ground finch species, the sharp-beaked ground finch *G. difficilis*, specialises on small seeds, insects and in some cases blood of other avian

species. Within the tree finches most species prey upon arthropods such as the large tree finch *C. psittacula*, the medium tree finch *C. pauper* and the small tree finch *C. parvulus*, the woodpecker tree finch *C. pallidus* and the mangrove finch *C. heliobates*. The sole member of the genus *Platyspiza* is the vegetarian finch *P. crassirotris* which feeds on flower buds. The relationship between bill shapes amongst the Finches and their environment (phenotype-environment) is clear. The speed of adaptive evolution within the finches over a short time scale has also been well documented (Vincek *et al*., 1997; Freeland & Boag, 1999; Sato *et al*., 2001).

Another clade which has provided extensive examples of trophic niche specialisation through morphological adaptation are the African cichlids of the African Great Lakes; Lake Malawi, Lake Tanganyika and Lake Victoria. Over 2,000 species are believed to have evolved over the last 10 million years (Kocher, 2004) with some faunas believed to diversified within the last 50 000 years (Kornfield, 1978). This rapid proliferation of diversity is manifested in differences in ecology, behaviour and reproductive biology (Fryer, 1972). Adaptive radiation of African cichlids, like Darwins finches, is attributed to exploitation of specific trophic niches from plankton and algae grazers to egg and fish predators (Turner, 1994). The incredible diversity found within African cichlids is believed to be facilitated, in part, by a key innovation associated with pharyngeal jaws (Hunter, 1998). One of the most well-studied groups are a monophyletic group of algae scrapers, known colloquially as mbuna *Tropheus*, *Simochromis* and *Petrochromis* spp. This diverse group feed on algae covered rocks and boulders and also breed in these habitats in relatively shallow waters (Ribbink *et al*., 1983; Konings, 1995; Konings, 2001; Genner & Turner, 2005; Ding *et al*., 2015). Genetic studies suggest a weak dispersal of mbuna can be attributed to extremely high site fidelity due to an inability to inhabit sand, sediment or deep waters (McKaye *et al*., 1983; Ribbink *et al*., 1983; Hert, 1992; Markert, 1999; Genner & Turner, 2005). Reproductive strategies within African cichlids are also highly variable, for example: oral incubation of eggs has evolved independently numerous times (Goodwin, 1998), while in other species, nest building and defence is commonly found, particularly in species inhabiting sandy substrates (McKaye *et al*., 1993).

1.6. Replicated Adaptive Radiation

A fascinating aspect of adaptive radiation is replicated adaptive radiations. This occurs when phenotypes adapted to a specific niche regularly appear or are repeated between different ecosystems. Two terms are often used interchangeably when referring to such occurrences in evolution - convergent and parallel. Convergent evolution is defined as the

appearance of similar phenotypes adapted to a specific niche between two unrelated species. Parallel evolution is the appearance of similar phenotypes adapted to a specific niche within a species, or from the same evolutionary origins. A classic example of replicated parallel adaptive radiation is the *Anolis* lizards of the Caribbean. *Anolis* lizards are small, diurnal lizards which are distributed across the Antilles, spending most of their time perched on different types of vegetation (Williams, 1972). On the larger islands of the Greater Antilles (Cuba, Jamaica, Hispaniola and Puerto Rico), anoles have repeatedly produced the same set of specialists adapted to a specific microenvironment, termed 'ecomorphs' or 'polymorphisms'. These polymorphisms share similar morphology, ecology and behaviour. The anoles inhabit four basic spatial niches on each island; ground, tree trunk, branches and twigs, all of which show a positive correlation with perch size and limb length (Irschick *et al*., 1997). The associated fitness consequences of such morphological differences were tested by transplanting lizards to small islands with limited vegetation types (Losos *et al*., 1997) and remarkably a shift in phenotype predicted by the correlation of perch size and limb length was observed over short generation times, which is likely due to high phenotypic plasticity (Losos *et al*., 2000; Schluter, 2000). An interesting question arising from replicated adaptive radiation is whether polymorphisms evolve once and distribute among ecosystems (allopatric), or evolve multiple times within a single ecosystem (sympatric) converging on similar morphologies (Gillespie, 2004). Within anoles the latter appears to be the case (Losos & Ricklefs, 2009); the majority of which have radiated independently from one another and yet have produced similar polymorphisms. The land snail genus *Mandarina*, on the Bonin Islands in the West Pacific for example, exhibit replicated adaptive radiation across a number of locations and like the anoles, through parsimonious phylogenetic reconstruction, show repeated and independent diversification of ecomorphs (Chiba, 2004). A third example of repeated adaptive radiation is the spiny leg clade of Hawaiian long-jawed spiders (Gillespie, 2004). After examination of DNA sequences, accrual of observed polymorphisms within an ecosystem arose through a combination of both dispersals from a single evolutionary lineage and convergent evolution of ecomorphs (Gillespie, 2004). The examples provided here support current thinking, that the emergence of ecomorphs occurs between closely related species (Chiba, 2004; Gillespie, 2004; Losos & Ricklefs, 2009; Losos, 2010). Such replicated adaptive radiations are not believed to be common (Losos, 2010) although an exception to this may be found in species of fish in post-glacial lakes.

1.7. Post-Glacial Lakes

Like Darwin's finches and African cichlids, many fish inhabiting post glacial lakes exhibit trophic specialisation within a species with a clear morphological polymorphism, and like the anole lizards, Mandarina land snails and Hawaiian long jawed spiders, these adaptive polymorphisms appear to be recurrent, commonly observed along the pelagic and benthic axis. What makes these polymorphisms of particular interest is they are repeated between closely related species and distantly related species, all of which exhibit common morphological characteristics and appear to have diverged in a relatively short time frame, given that most lakes in the Northern Hemisphere were created during the last glacial retreat less than 15,000 years ago. In addition to this, small, but significant, genetic differences between some sympatric polymorphisms has been observed - these examples feature a variety of fish taxa, however they are found predominately within the salmonids (salmon, trout, whitefish) (Schluter, 1996). The genetically distinct sympatric polymorphisms observed today may have diverged rapidly, if diversification occurred in sympatry. However, determining the origins of observed polymorphisms in post glacial freshwater lakes is complex. The invasion routes for anadromous ancestral fish from glacial refugia were accessible sporadically and may have been available once (single invasion) or several times (multiple invasions) (Behnke, 1972; McKeown *et al*., 2010) combined with man-made transplants of fish, the genetic integrity of observed fish communities today may mask the true origins of some populations (Hindar *et al*., 1991). However, given the availability of numerous depauperate and heterogeneous freshwater lake environments, the number of documented replicated polymorphisms and the phenotypic plasticity observed in a number of freshwater fish species, freshwater post-glacial lakes are ideal study systems to examine the potential early mechanisms of divergence and ecological speciation.

1.7.1. Three-Spined Sticklebacks

Three-spined sticklebacks *Gasterosteus* have a Holarctic distribution and are found in both marine and freshwater systems across North America, Europe and Asia (Ostlund-Nilsson *et al*., 2006). They provide an ideal model species to study ecological speciation as populations have consistently diverged across many phenotypic axes (McKinnon & Rundle, 2002). Three-spine stickleback polymorphisms exist between lakes and stream systems (Lavin *et al*., 1993), anadromous and freshwater life cycles (Rafiński *et al*., 1989) and benthic and limnetic zones (McPhail, 1994). Benthic and limnetic three-spined sticklebacks are well studied polymorphisms - usually found as a species pair in a single lake, which exhibit variation in morphology, foraging behaviour and genetic composition (Schluter &

McPhail, 1993; Day *et al*., 1994; Nagel & Schluter, 1998; Piechel *et al*., 2001). Benthic sticklebacks possess short stout bodies, with wide mouths and few small gill rakers, shown to aid foraging ability on benthic invertebrates (McPhail, 1984, 1992). In contrast, limnetic three-spine sticklebacks have long bodies, pointed mouths and many long gill rakers, demonstrated to aid filtration of small zooplankton from the water column (McPhail, 1984, 1992). In addition to this, benthic and limnetic polymorphisms exhibit variation in defensive body armour; benthic sticklebacks often have reduced or no dorsal spines, and reduced numbers of lateral plates, compared with limnetic fish which retain dorsal spines and lateral plates (McPhail, 1992, 1994; Peichel *et al*., 2001). This variation in defence anatomy is believed to be the result of differential predation pressures as the morphs feed in distinct habitats of a lake system. Limnetic sticklebacks, which operate in pelagic open waters, are predated upon by birds and fish, thus large spines act as a useful deterrent (Peichel *et al*., 2001). Benthics, which operate in near-shore areas, are frequently predated by invertebrates, therefore dorsal spines would be a less effective defence (Peichel *et al*., 2001). Studies have investigated the genetic components associated with polymorphic three-spine sticklebacks (Ridgway & McPhail, 1984; Peichel *et al*., 2001 Reusch *et al*., 2001). Most suggest observed polymorphisms are young, within the last 13 000 years (McPhail, 1984; Taylor *et al*., 1997). What is clear from the literature is the potential fragility of polymorphic populations. In Lake Enos for example, the presence of a 'hybrid swarm' has been reported (Taylor *et al*., 2006). The genetic integrity of benthic and limnetic polymorphisms once found here appears to have collapsed, reverting back to a single population, in the absence of strong reproductive barriers. The introduction of an exotic catfish, the brown bullhead *Ameiurus neblusosus* is believed to have coincided with the collapse of the two trophic polymorphisms in Lake Enos (Taylor *et al*., 2006). The factors driving the production of these morphs have important implications for evolution and ecological speciation.

1.7.2. Rainbow Smelt

Rainbow smelt *Osmerus mordax* have a circumpolar distribution however, they are native to the Atlantic coast of North America (Scott & Crossman, 1973). Like sticklebacks, there are anadromous and lacustrine populations of rainbow smelt, the latter of which have diversified into different polymorphisms (Taylor & Bentzen, 1993). Lacustrine polymorphisms commonly manifest as 'dwarf' and 'normal' smelt (Nellbring, 1989). Dwarf smelt share similar morphological features to limnetic ecotypes in other species for example; they have large eyes, an increased number of narrow gill-rakers and small mouths (Taylor & Bentzen, 1993), compared with 'normal' smelt. Such differences in morphology are believed to be functionally important and reflect smelt foraging behaviour (Taylor &

Bentzen, 1993). Interestingly, in addition to morphological differences, in Lac Saint-Jean, Quebec, Canada, dwarf and normal rainbow smelt exhibit substantial variation in life history traits such as growth, length at age, age of maturity and reproductive strategy (Saint-Laurent *et al*., 2003). Dwarf smelt grow to a much smaller size and are smaller at the same age as normal smelt, they mature earlier and are also semelparous (complete a single reproduction event before dying), compared with normal smelt which are larger at the same age, mature later and are iteroparous (multiple reproductive cycles over a lifetime) (Saint-Laurent *et al*., 2003). The clear differences in age structures found between the two ecotypes suggests higher mortality in dwarf populations compared with normal populations (Saint-Laurent *et al*., 2003), which may be indicative of disparate predation pressures, similar to that observed in three-spine sticklebacks (Peichel *et al*., 2001).

1.7.3. Lake Whitefish

Another salmonid, the whitefish *Coregonus* spp. are distributed across North America *C. clupeaformis* (Scott & Crossman, 1973; Bernatchez & Dodson, 1991) and Europe *C. lavaretus* (Svärdson, 1953, 1979). Many sympatric ecotypes have been described exhibiting differences in morphology, meristic traits, food selection, growth rates and functional genes (Bernatchez *et al*., 2010). In Lake Muddusjärvi, Finland, three sympatric ecotypes have been described (Kahilainen & Østbye, 2006). Each ecotype can be classified by gill-raker length which has been positively correlated with trophic position. Two benthic forms, both with sparse gill-rakers known as large and small benthics feed in the littoral and profundal zone predominately on macroinvertebrates and zoobenthos, respectively (Kahilainen & Østbye, 2006). A third, densely gill-rakered form inhabits pelagic habitats and feeds predominately on surface insects. All three ecotypes display very little dietary overlap suggesting temporal stability over time (Kahilainen & Østbye, 2006). Aside from gill-rakers, differences in head and pectoral fin traits have been identified; important in swimming ability and foraging efficiency (Janssen, 1980; Webb, 1984; Kahilainen & Østbye, 2006). In North America many benthic and pelagic *C. clupeaformis* forms, often referred to as 'dwarf' (limnetic) and normal (benthic) species pairs have been described in a number of lakes (Rogers *et al*., 2002; Rogers & Bernatchez, 2007; Fraser *et al*., 2011). Extensive genetic investigation has revealed a number of important functional genes associated with each ecotype. Remarkably, limnetic 'dwarf' forms consistently exhibited significant over-expression of genes related to energy metabolism - lipid metabolism and detoxification, believed to enhance an individual's survival (Fraser *et al*., 2011) compared with benthic 'normal' forms which exhibited an upregulation of genes associated with growth (cell cycle and cell growth, protein synthesis). These genes were not only found

within species pairs, but were consistently found in parallel with dwarf and normal whitefish across multiple lakes (Fraser *et al*., 2011). Thus, the expression of such genes appears to be a trade-off associated with use of alternative habitats within a single ecosystem.

1.7.4. Atlantic Salmon

Atlantic salmon *Salmo Salar*, occur naturally across north western Europe, Iceland, Greenland and eastern North America (MacCrimmon & Gots, 1979). Most populations are anadromous, however a few landlocked populations have been described (Ståhl, 1987; Tessier *et al*., 1997; Tessier & Bernatchez, 1999). Despite large marine migrations, sometimes several thousand miles, the strong innate homing ability of *S. salar* to natal freshwater streams and rivers (Stabell, 1984; McIsaac & Quinn, 1988) where they reproduce, has resulted in little gene flow between populations and thus resulted in extensive population structuring and local adaptation (Taylor, 1999; King *et al*., 2001; Dionne *et al*., 2008). Populations of *S. salar* exhibit alternative morphologies and life history strategies, particularly with age and size of maturity (Simpson, 1992; Metcalfe, 1998;). In some *S. sala*r populations, differences in flow regime have been shown to affect the size of pectoral fin and body shape in juvenile *S. salar* (Riddel & Leggett, 1981). These morphological adaptations are considered to increase and maximize efficiency in juvenile swimming behavior and are inherited from parents (Riddel *et al*., 1981). Maturity in *S. salar* is of particular interest as many different strategies exist within the species. The most common reproductive cycle involves *S. salar* smolting, a process in which the internal physiology and external morphology of juvenile *S. salar* modifies to survive saline waters. Differences in growth and lipid thresholds have been shown to influence what year a juvenile will smolt, as juveniles with high growth and lipid levels are more likely to migrate to marine waters than juveniles with low growth and low lipid levels (Thorpe, 1977; Wright *et al*., 1990; Simpson, 1992; Jonsson & Jonsson, 2003) and is thus dependent on an individual's ability to acquire food and grow (Metcalfe, 1998). A similar lipid threshold has been described in mature *S. salar* returning to natal streams to spawn from the Batlic sea (Thurow, 1966). Returning mature adults require a minimum lipid level of about 12% (in Baltic salmon) to invest in egg production (Kadri *et al*., 1996) and thus delay reproduction unless an appropriate level of lipid is attained (Thurow, 1966; Thorpe, 1994). The time taken for *S. salar* to return to spawn is reflected in their classification as either single sea-winter fish or multi-sea winter fish (Niemelä *et al*., 2006; Vähä *et al*., 2007). Finally, precocious parr represent another important variant of *S. salar*. These small male *S. salar* grow to the size of juvenile *S. salar* but fully mature in freshwater, again influenced by mesenteric fat (Rowe *et al*., 1991). Precocious parr represent an important part of the *S. salar* population and have

been found to contribute extensively to successful reproduction (Fleming, 1996; Morán *et al*., 1996; Garcia-Vazquez *et al*., 2001).

1.7.5. Arctic Charr

Arctic charr have a northern circumpolar distribution (Behnke, 1980; Johnson, 1980). Across this range many charr polymorphisms co-exist, exhibiting differences in colouration (Hindar & Jonsson, 1982; Smalås *et al*., 2013), internal and external morphology (Snorrason *et al*., 1994; Adams *et al*., 1998), behaviour (Jonsson & Hindar, 1982; Skúlason & Smith, 1995), geographical and temporal variation in spawning (Sandlund *et al*., 1992) and life history traits such as growth rate and age of maturity (Skúlason *et al*., 1996). The numerous and often stark variation in British and Irish populations alone led to at least 15 'species' of charr being described historically (Adams & Maitland, 2007), however these forms are commonly viewed as polymorphisms which belong to a single species, *Salvelinus alpinus* (Jonsson & Jonsson, 2001). The so called 'charr problem' described by Jonnson and Jonsson (2001), pertains to the issue of charr classification, particularly in view of the biological species concept (Mayr, 1965). Genetic investigation suggests most co-existing polymorphisms are more closely related to each other than similar morphs inhabiting different locations (Hindar *et al*., 1986). Thus, these polymorphisms are believed to have arisen sympatrically and in parallel and are considered examples of ecological speciation. Given the evolutionary significance of such sympatric divergence, the examination of the genetic, ecological, physiological and behavioural variation within sympatric polymorphisms of charr has been extensive.

Similar to three-spined sticklebacks, many charr polymorphisms exist across the benthic/limnetic axis, however three and sometimes four sympatric polymorphisms have been described in a single lake, such as Lake Thingvallavatn, Iceland. Lake Thingvallavatn is a large, land-locked volcanic lake which formed around 10 000 years ago. Interestingly, four charr morphs have been described here; a small benthic (SB), a large benthic (LB), a planktivore (PL) and a piscivore (PI) morph (Skúlason *et al*., 1989). Each morph is believed to have formed in sympatry adapted to a particular trophic niche, reflected in their external morphology. For example, small and large benthic morphs have short lower jaws with blunt snouts compared with planktivorous and piscivorous morphs which have evenly protruding jaws and pointed snouts (Skúlason *et al*., 1989).

1.7.6. Brown Trout

Brown trout *Salmo trutta* exhibit extensive plasticity in morphology, behaviour, ecology and life history. Such wide phenotypic variation led early taxonomists to classify

numerous species of brown trout (Gunther, 1866; Behnke, 1986; Ferguson, 1989). However, today most are classified as part of the single polytypic species *S. trutta* (Ferguson, 1989). Although considered a single species, confusingly many vernacular names are still used to describe the multiple life histories available to *S. trutta*. The ability of *S. trutta* to inhabit almost all aquatic environments has made it one of the most invasive species in the world (Klemetsen *et al*., 2003). Within each diverse habitat *S. trutta* inhabits, from coastal and estuarine waters to freshwater lakes and river, they express a gradient of adaptive phenotypes (Klemetsen *et al*., 2003).

S. trutta can be split into two broad categories - either resident or migratory. Resident *S. trutta* are often referred to as resident or stream-resident brown trout. These resident *S. trutta* complete their entire life cycles in natal streams and remain relatively small their entire lives. Migratory *S. trutta* can be split further into anadromous and potamodromous *S. trutta*.

Like *S. salar*, *S. trutta* can adopt an anadromous life history, often referred to colloquially as sea trout. Juvenile anadromous *S. trutta* undergo smoltification, the process in which an organism's internal physiology, behaviour, colouration and external morphology changes to prepare for migration to saline waters, before returning to natal streams as mature adults to reproduce. During this process anadromous *S. trutta* are referred to as smolts; they lose their dark colouration and become silvery or mirrored, a common adaptation to avoid detection (crypsis), in marine pelagic habitats (Johnsen & Sosik, 2003). The mechanisms driving anadromy in *S. trutta* are not fully understood but evidence suggests a combination of prey availability, nutrition thresholds and genetics can contribute to a juvenile *S. salar* and *S. trutta* smolting or not (Simpson, 1992; Jonsson & Jonsson, 2002; Van Leeuwen *et al*., 2016). Interestingly, the 'decision' to smolt is not uniform, indeed in a single population of anadromous *S. trutta* the age of smolts ranged from 2-7 years old (Jonsson, 1985) and mature anadromous *S. trutta* like *S. salar* can spend a single or multiple growing season at sea before returning to spawn, often referred to as single-sea winter or multi-sea winter fish. When mature anadromous *S. trutta* do return to spawn they are often large and as a result are more fecund than *S. trutta* that matured in freshwater (Elliott, 1995; Van Leeuwen *et al*., 2016). Anadromous *S. trutta* are more fecund due to their larger body volume and the amount of energy available for egg production (Jonsson & Jonsson, 1997; Klemetsen *et al*., 2003).

Some anadromous *S. trutta* that have been described partially migrate into brackish estuarine waters before returning to spawn. Colloquially these short distance migrants have been referred to as estuarine, slob and tidal trout (Campbell, 1977; Elliott, 1984; McCarthy & Waldron, 2000; Etheridge *et al*., 2008). These forms are relatively understudied, however

they are believed to actively feed between the estuarine and freshwater zones of an outflowing river, and can grow to large size (Kennedy & Fitzmaurice, 1971).

Potamodromous *S. trutta* are perhaps the most common form of *S. trutta*, completing their entire life cycle in freshwater systems. Potamodromy itself can be divided into two categories - either adfluvial potamodromy and fluvial potamodromy. Fluvial potamodromous *S. trutta* are trout that migrate from natal streams into larger rivers and remain between these two systems for their entire life cycle, compared to adfluvial potamodromous *S. trutta* which migrate to lacustrine systems to mature. Adfluvial *S. trutta* (referred to as lacustrine *S. trutta* hereafter) are sometimes referred to colloquially as lake trout but often retain the name brown trout, as do fluvial *S. trutta*. Most lacustrine *S. trutta* are benthivorous, feeding in the littoral zone on macro-benthos and reach sexual maturity around 4-5 years of age (Klemetsen *et al*., 2003). Like numerous other post-glacial species however, lacustrine *S. trutta* populations exhibiting adaptive trophic phenotypes have been described, mainly segregated into benthic, pelagic and piscivorous niches (Ryman *et al*., 1979; Ferguson & Taggart, 1991).

Table 1.1. Publications from north-western Europe covering various topics on ferox trout.

Variable/ Topic	Locality	References
Distribution	Various lakes, Scotland	Campbell, 1971;1979
	Various lakes, Scotland	Greer, 1995
Morphology	Lough Melvin, Ireland	Cawdery & Ferguson, 1988
Growth	Various lakes, Scotland	Campbell, 1971; 1979
	Various lakes, Ireland	Kennedy & Fitzmaurice, 1971
	Various lakes, Ireland	Ferguson & Mason, 1981
Maturity	Various lakes, Scotland	Mangel & Abrahams, 2001
Longevity	Various lakes, Ireland	Ferguson & Mason, 1981
	Various lakes, Scotland	Mangel & Abrahams, 2001
Trophic level	Various lakes, Norway	L'Abee-Lund *et al*., 1992
	Loch Ness, Scotland	Grey, 2001
	Loch Ness, Scotland	Grey *et al*., 2002
Genetics	Lough Melvin, Ireland	Ferguson & Mason, 1981
	Various lakes, north-western Europe	Ferguson, 1989
	Various lakes, Ireland	Hamilton *et al*., 1989
	Various lakes, Scotland	Stephen & McAndrew, 1990
	Lough Melvin, Ireland	Ferguson & Taggart, 1991
	Various lakes, north-western Europe	Hynes *et al*., 1996
	Loch Awe , Scotland	Duguid *et al*., 2006
	Loch Laggan, Scotland	Duguid *et al*., 2006
	Various lakes, Ireland and Scotland	McKeown *et al*., 2010
Movement	Loch Rannoch, Scotland	Thorne *et al*., 2016
Conservation	Various lakes, Ireland	Ferguson *et al*., 2004

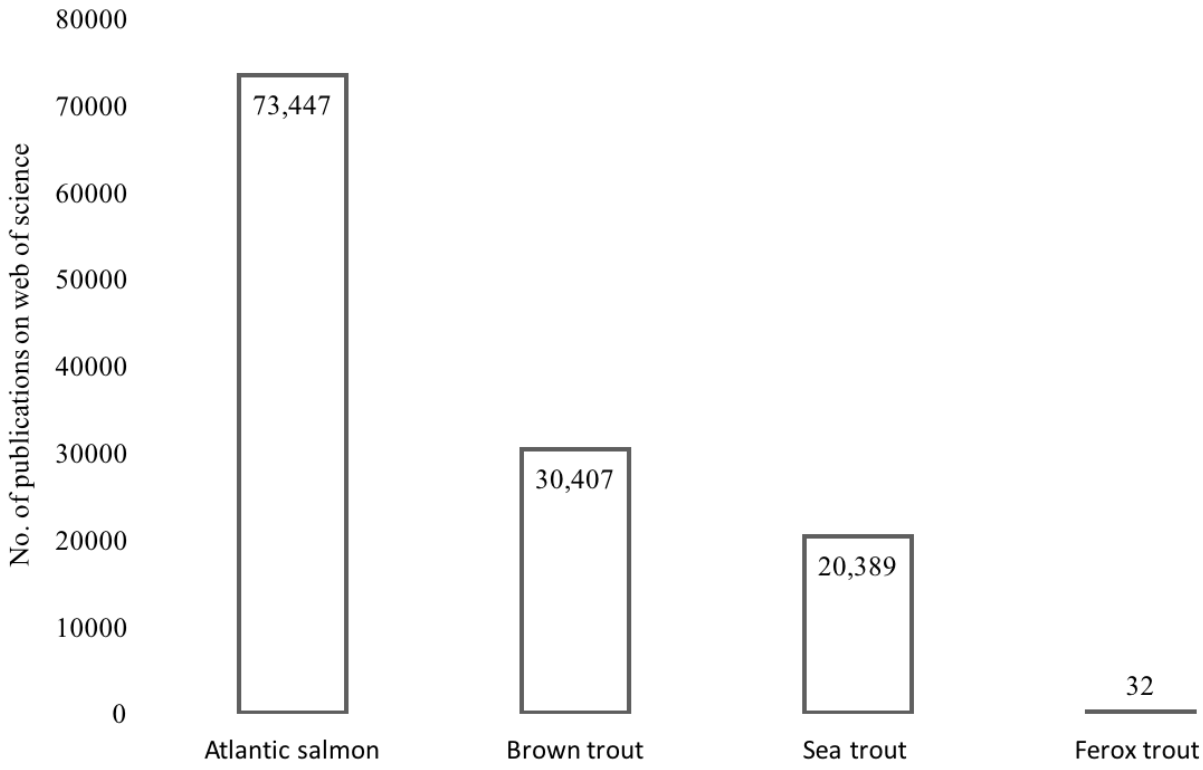


Figure 1.2. Publication records of ferox trout from the web of science database

Although once considered multiple species, polymorphisms of *S. trutta* observed today are considered adopted life history strategies of the polyphenic brown trout species complex (Wheeler & Du Heaume, 1969). The interest in some of the life history traits goes beyond taxonomic classification, however. Given the commercial value of the anadromous brown trout (sea trout) they have received a disproportionate amount of attention from the scientific community, mainly to determine the underlying mechanisms involved in producing anadromous populations to increase returning numbers of mature adults, subsequently targeted by game anglers. One life history strategy which has received very little attention from the scientific community is the large, long-lived, piscivorous lacustrine brown trout, often referred to as ferox trout (Fig. 1.2.; Table 1.1.).

1.8. A Brief History Of Ferox Trout

The first detailed description of ferox trout in Scotland came from Campbell in March 1971. Here Campbell describes ferox trout as 'exceptionally large, and old, trout ('ferox') characteristic of large, soft water lochs, but which in fact only represent a fraction of the trout population present'. He also notes the capture of large old non-maturing trout, suggesting potential delayed maturation in large trout. In July of the same year Kennedy & Fitzmaurice (1971) similarly describe ferox trout from Irish waters as very large, fast

growing, predatory trout feeding mainly on smaller trout, salmon parr and smolts, perch and other fishes and are found inhabiting large lakes. Kennedy & Fitzmaurice also comment on the lack of specimens available for scientific examination given the rarity of ferox trout captures. Campbell (1971) and Kennedy & Fitzmaurice (1971) describe important qualities associated with ferox trout. They are large piscivorous predators; have extended longevity; are associated with large, soft water, oligotrophic lakes and are rare. Although no evidence is provided explaining how these characteristics arise or contribute to the maintenance of ferox trout populations, Kennedy & Fitzmaurice (1971) pose important hypotheses for future studies: 'Does the change-over to a pike-like diet occur as a matter of necessity when these trout – which have a faster-than-average growth even when young – begin to find available supplies of invertebrate food insufficient to satisfy their hunger? Or are they naturally aggressive individuals which become predatory while still young? And if so, does heredity enter the picture? At this point, there are no answers to these questions.' These questions are still not fully answered to this day.

Following his paper in 1971, Campbell produced a review paper on ferox trout which attempted to explore some of the questions raised 8 years previously. Campbell (1979) examined 141 ferox trout from 22 lochs in Scotland, collected over two decades. Through back-calculation of scales Campbell (1979) investigated the length-at-age relationship of ferox trout and found ferox trout grow slowly until reaching a critical length of about 30cm and then begin to grow much faster and live longer than 'normal' brown trout. Campbell also makes a distinction between ferox trout growth and fast growing brown trout living in eutrophic waters, as such brown trout do not reach the same dimensions or age as ferox trout. Importantly Campbell also investigated fine scale environmental characteristics associated with the distribution of ferox trout and states three essential conditions are required for ferox trout to occur in the wild: (1) oligotrophic waters; (2) the presence of Arctic charr; and (3) a large loch (over 100 ha in extent). These predictions were made on the basis of 113 Scottish lakes 58 of which had a ferox trout population. This was an important finding, providing evidence that the distribution of ferox trout populations, at least in Scotland, is not random but associated to measurable environmental parameters. Ferox trout are also described as bearing a superficial resemblance to fresh run salmon with relatively few black spots on a silver background with males, particularly older specimens, often lean and possessing disproportionally long heads, large and obvious teeth with pronounced kypes. Campbell (1979) also discusses the history of ferox trout taxonomy describing their earlier classifications. Ferox trout were classified as *Salmo lacustris* in 1789 by Berkenhout, which was later changed to *Salmo ferox* by Jardine (1835), which was later accepted as the species

name by Günther (1880). By 1969, following taxonomic revisions, many unique classifications of British trout were eliminated, including *Salmo ferox* and subsequently ferox trout were recognised only as one of the many forms of a single species, *Salmo trutta* (Wheeler & Du Heaume, 1969). The taxonomic classification of ferox trout became an important theme of subsequent research.

Ferguson & Mason (1981) were the first to investigate the genetic composition of ferox trout by examining allozyme variation of three brown trout forms exhibiting discrete phenotypes known vernacularly as gillaroo, sonaghen and ferox. Electrophoretic studies found the three morphotypes represented genetically, reproductively isolated populations. Ferox trout specifically were characterised by a very high frequency of the lactate-dehydrogenase (LDH-5) '105' allele. Ferguson & Mason (1981) suggest this allele may contribute to the observed growth and age potential of ferox trout. They also speculate the origin of ferox trout by making divergence estimates. Interestingly, the divergence of gillaroo and sonaghen occurred some 40 000–65 000 years ago, however ferox trout are reported occurring 230 000 – 265 000 years ago and thus represent a much older and distinct lineage from the other two forms. As a result, Ferguson & Mason (1981) discuss the taxonomic discrepancy that exists around ferox trout, noting some authors regard ferox trout as simply large brown trout. Thus, they conclude that, in Lough Melvin at least, the brown trout population there is diverse and perhaps a rare example of what was potentially a widespread occurrence across Ireland and as such this has implications for how we view unique populations from an ecological and fishery management perspective. The authors state that the conservation of such populations is of 'intrinsic interest'.

Cawdery & Ferguson (1988) follow the initial investigation of Lough Melvin by providing the first statistical analysis of head shape morphology of all three morphotypes, including ferox trout. Ferox trout, referred to as an 'ancestral' trout lineage were successfully assigned to a single group by stepwise discriminant analysis, measuring whole body characters including: fork length, standard length, body depth, caudal peduncle, post dorsal fin, post anal fin, head length, head depth, snout length, orbit diameter, jaw length, pectoral fin, pelvic fin, caudal fin, dorsal fin, anal fin, dorsal fin base, anal fin base, and longest gill-raker. Long held opinions on visual descriptions of ferox trout were thus now verified using principal component analysis and stepwise discriminant analysis. Cawdery & Ferguson (1988) describe ferox trout as having long heads, short anal fins, deep heads, short pectoral fins, short dorsal fins, narrow bodies, short caudal fins and short gill-rakers. This result further supported the evidence suggesting ferox trout are indeed distinct both genetically and phenotypically in Lough Melvin.

By 1989, Irish salmonid research began to focus on 'How did each unique morphotype arise?' Principally the research centred on the evolution of these populations since the last glaciation in north-western Europe. Hamilton *et al*., (1989) examined samples from 62 discrete drainage systems across Ireland, Britain, Iceland and Spain and concluded the LDH-5 '100' allele, synonymous with the '105' allele previously reported by Ferguson & Mason (1981) was indeed an ancestral allele, as it is present in 11 other salmonid species. In contrast, gillaroo and sonaghen trout were characterised by LDH-5 '90' believed to have replaced the ancestral '100' allele which represents a late-arriving modern race of trout. Thus, Hamilton *et al*., (1989) concludes that at least two post-glacial colonisation events occurred in north-west Europe and suggests, with specific reference to ferox, that if a particular niche exists (i.e. piscivory) then it may be possible for ancestral and modern lineages to co-exist. In the same year Ferguson (1989) produced an extensive review of genetic variation of brown trout populations from the USA, Iceland, Ireland, Britain, France, Finland, Sweden, Norway, Greece and the U.S.S.R.. Ferguson asserts ferox trout as an ancestral lineage, but does not discount that out-with Lough Melvin, large brown trout, referred to as ferox, necessarily have a genetic basis.

This was indeed found to be the case in 1990, when 34 enzyme loci including the lactate dehydrogenase (LDH) allele from Scottish *S. trutta* were screened by Stephen & McAndrew (1990). They found no fixed association between ferox trout and LDH-5 '100' and suggest ferox trout are the result of enhanced feeding opportunities particularly in areas where *S. alpinus* thrive. It was reported in Loch Rannoch however, that some genetically isolated populations of *S. trutta* may exist, with particular reference to the ferox trout sampled there, although the evidence was not clear.

In 1991, Ferguson & Taggart (1991) produced a thorough examination of Lough Melvin trout through a combination of lake sampling and hatchery stocks of gillaroo and sonaghen. It was noted insufficient numbers of ferox trout were obtained to create a hatchery and some of the lake-caught ferox sub-samples were too small to analyse. This was the first real evidence, highlighting the difficulty in acquiring adequate ferox trout samples since it was first suggested by Kennedy & Fitzmaurice (1971). Ferguson & Taggart (1991) provide further evidence supporting the ideas of an ancestral LDH-5 '100' lineage and modern LDH-5 '90' lineage persist in the lake, however they categorically state that given such clear genetic and morphological evidence, all three morphotypes should revert back to using nineteenth century sub-species names. Thus, gillraroo should be designated *Salmo trutta stomachius* Gunter, 1866, sonaghen *S. t. nigripinnis* Gunter, 1886 and ferox as *S. t. ferox* Jardine & Selby, 1835. By proposing re-classification as distinct sub-species, Ferguson &

Taggart (1991) hoped that the value of conserving and appropriately managing these discrete populations would be recognised.

A paper of note, out-with Ireland and Scotland at this time is L'Abee-Lund *et al*., (1991). L'Abee-Lund *et al*., (1991) examined piscivory in *S. trutta* and *S. alpinus* in 13 lakes across central Norway, referring to literature from Britain and Ireland, particularly Campbell (1979). The focus of this study was to determine length thresholds at which *S. trutta* and *S. alpinus* become piscivorous and the composition of their diets. The study produced 4 major outcomes: (1) piscivory in *S. trutta* and *S. alpinus* is rare, only a fraction of the main population become piscivorous; (2) Both *S. trutta* and *S. alpinus* in the Norwegian lakes sampled preyed on smaller fish species such as three-spined sticklebacks *Gasterosteus aculeatus* and minnow *Phoxinus phoxinus*; (3) *S. trutta* and *S. alpinus* became piscivorous about a length of 13-20cm. Interesting L'Abee-Lund *et al*., (1991) found piscivorous *S. trutta* fed predominately on non-salmonids, with one exception where they were found to feed on *S. alpinus*. *S. trutta* began feeding on *G. aculeatus* and *P. phoxinus* at approximately 13-15cm and *S. alpinus* at approximately 20cm - much shorter than the Campbell (1979) threshold of 30cm before a switch to piscivory in Scottish ferox trout. L'Abee-Lund *et al*., (1991) also report large *S. trutta* did not feed exclusively on *S. alpinus* but continued to supplement their diet with *G. aculeatus* and *P. phoxinus*. Unlike most publications from Ireland and Scotland, L'Abee-Lund *et al*., (1991) refrain from referring to large *S. trutta* as ferox trout, only as facultative piscivores. They do however acknowledge the importance of piscivorous *S. trutta* as predators in a lacustrine system in the maintenance of other fish communities.

In 1990, ferox trout are mentioned in Finch's book 'Longevity, senescence and the genome – pp. 142-143'. Finch (1990) notes the longevity achieved by ferox trout, some reaching at least 20 years of age, weighing thirty-two pounds (14.5 Kg) and 90 cm in length. Finch (1990) states that although a potential contributing factor, LDH-5 '105' alone could not account for the growth and longevity observed in wild populations. Finch (1990) importantly highlights a lack of information on age-related mortality, something that was later reaffirmed by Elliot (1994). Finch (1990) proposes the observed growth patterns in ferox trout are undoubtedly related to extended life spans, which contradicts many other species that achieve extended life span through diet restriction.

In Scotland, the unanswered questions surrounding ferox trout prompted the formation of a dedicated group of anglers and scientists in 1985. The aptly named Ferox85 group began collaborating with scientists providing much needed assistance with specimens for scientific examination. In 1995, a founding member of the group produced an accessible

synopsis describing ferox trout ecology, conservation and methods of capture. Greer (1995), discusses the taxonomic debate, ferox trout prey, their environment, growth and longevity and conservation strategies. Greer (1995) highlights the narrow range of species available to ferox trout in Scottish lakes compared with Scandinavian and mainland European populations, given the relatively small amount of fish species that successfully colonised Scottish lakes after deglaciation. Greer (1995) confirms the importance of *S. alpinus* in ferox trout diets, however concedes other *S. trutta*, European perch *Perca fluviatilis* and even small mammals such as voles (*Microtus* spp.) have been found in the stomachs of ferox trout. Greer (1995) subscribes to the conventional model of ferox trout growth proposed by Campbell (1979) in which they are initially slow growers before reaching a critical length threshold of 30-35cm before becoming piscivores. Importantly, Greer (1995) appeals to a wider audience of stakeholders, mainly anglers and fisheries scientists, in an attempt to pull resources to further enhance the understanding of this cryptic fish.

By 1996, advances in mitochondrial DNA (mtDNA) analysis allowed further examination of *S. trutta* in post-glacial lakes across north western Europe. With particular reference to the Lough Melvin, Hynes *et al*., (1996) investigated the mtDNA of numerous *S. trutta* populations. Hynes *et al*., (1996) acknowledge the importance of previous investigations of enzyme loci, however mtDNA analysis had a significant advantage when studying population phylogeny. As previously reported in the literature (Cawdery & Ferguson, 1988; Hamilton *et al*., 1989; Ferguson, 1989; Ferguson & Taggart, 1991), the identification of modern and ancestral lineages suggested at least two colonisations events occurred in Lough Melvin. The issue with nuclear DNA genes for assessing colonisation patterns is that secondary contact of another population may result in loss of nuclear divergence due to gene flow and recombination. As mtDNA is inherited asexually however, individuals can be organised into matriarchal lineages and thus dendrograms based on cluster analysis and divergence can be constructed. A significant discovery by Hynes *et al*., (1996) was the colonisation of Lough Melvin by three independent lineages of *S. trutta*. Thus, the invasion of *S. trutta* in north western Europe was proposed as more complex than the dual invasion theory previously suggested. In the same year, Mangel (1996) attempted to address the lack of information regarding age-related mortality suggested by Finch (1990) and Elliot (1994), by constructing theoretical growth curves to examine age at maturity and the effects of mortality on ferox trout. The main issue Mangel (1996) tried to address was, 'how could ferox trout escape the size ceiling that restricts other *S. trutta*?' Numerous mathematical models were constructed with biological variables such as size dependent mortality and projected growth rates, using the Von Bertalanffy growth equation. Some models allowed

for the production of large but not old trout. The only model that successfully produced ferox trout growth and longevity was modified so that mortality rate decreased as size increased; only then could the production of large, old trout be achieved. Mangel (1996) concludes more variation derived from life history characteristics may provide biological variables useful for further analysis.

In 2001, Grey (2001) examined the trophic ecology of *S. trutta* in Loch Ness, Scotland. Traditionally, ferox trout diet was determined by stomach content analysis (SCA). Although useful, SCA is limited for a number of reasons, mainly that prey in stomach contents represent a snapshot of prey ingested immediately prior to sampling, and thus may be empty or reflect a small range of prey targeted by the species. Secondly, prey that has been ingested may not necessarily be assimilated into tissues and thus contribute to an individual's overall growth and fitness. A more accurate measure of food assimilation can be obtained through stable isotope analysis. By examining two stable isotopes $\delta^{13}C$ and $\delta^{15}N$, the importance of different food sources can be determined. The examination of $\delta^{15}N$ is of particular interest in trophic studies, as $\delta^{13}C$ gives an indication of where the food has came from i.e. marine, estuarine or freshwaters, $\delta^{15}N$ signatures indicate the trophic level an animal operates at. Thus, increasing levels, or higher $\delta^{15}N$ indicates a movement up the food web. Grey (2001) reported elevated levels of $\delta^{15}N$ in ferox trout when compared with other non-piscivorous *S. trutta*, providing evidence that ferox trout are indeed apex predators within a lacustrine system. In addition to this Grey (2001) investigated the relationship between *S. trutta* length and stable isotope ratios. Grey (2001) found a positive relationship between $\delta^{15}N$ and increasing body length. Although Grey (2001) mentions the special circumstances reported in Lough Melvin, it is made clear that there is no evidence of genetic divergence between ferox trout in Loch Ness and sympatric *S. trutta*. This important study helped give ecologists another tool to confirm collected ferox trout specimens are indeed feeding at different trophic levels from sympatric *S. trutta*.

In a follow up to Mangel (1996), Mangel and Abrahams (2001) attempt to identify the role that ecological and biochemical adaptations play in the longevity of ferox trout. To achieve this Mangel and Abrahams (2001) constructed an elaborate model using multiple variables including environmental parameters such as time of year, daylight hours, littoral volume, benthic volume and littoral volume. Physiological parameters such as weight, length, growth rate and much more. Using stepwise multiple logistic regression Mangel & Abrahams (2001) determined that a model containing only three parameters explained 92.5% of variation, regarding the existence and number of ferox trout within a *S. trutta* population. These parameters were: (1) volume of littoral zone; (2) volume of benthic zone;

and (3) mortality rate (dependent and independent). Mangel and Abrahams (2001) propose the volume of littoral zone and the production of ferox trout is presumably linked to territory size available to ferox trout and thus food access. The relationship between volume of benthic zone was less clear and actually provided little explanatory power. Importantly after numerous simulations the model again highlighted the rarity of ferox trout, with just under 6% of the total *S. trutta* population becoming ferox trout.

In 2004 the appeal to recognise the biodiversity present in Lough Melvin and beyond is renewed by Ferguson (2004), stating their considerable genetic, morphological and ecological distinctness warrants more than sub-species status for the *S. trutta* as was previously suggested, and in fact they should be designated as separate species in their own right. Ferguson (2004) underscores the urgency of appropriate designation by describing relevant and pressing dangers facing local communities of distinct *S. trutta*. Namely the introduction of non-native species such as the zebra mussel *Dreissena polymorpha*, rudd *Scardinius erythropthalmus* and rudd/roach hybrids. Ferguson (2004) also warns against the dangers of anthropogenic stocking which can result in competitive exclusion of native fish and the risk of interbreeding which can cause loss of genetic integrity. Ferguson (2004) concludes that the designation as a sub-species is no longer useful, as conservation bodies fail to recognise sub-species and thus do not provide adequate protection of the discrete Melvin trout groups and proposes community-based conservation strategies are required to ensure appropriate protection of biodiversity.

In 2006, *S. trutta* from two lakes in Scotland, Loch Awe and Loch Laggan were sampled and the genetic diversity investigated by examining variation in allozymes, mtDNA and microsatellites. Duguid *et al*., (2006) discovered ferox trout from both lakes were reproductively isolated and genetically distinct from sympatric *S. trutta*. Remarkably, the ferox trout from Loch Awe, Laggan and Melvin are derived from a common ancestral mtDNA lineage and are thus more genetically similar to each other than sympatric *S. trutta*. Duguid *et al*., (2006) reiterate Fergusons (2004) plea to recognise ferox trout as a distinct species however also agree conservation should be community led and not based entirely on taxonomic classification.

In 2008, based on the substantial research conducted by Ferguson *et al*., (1981-2006), Lough Melvin trout were formally recognised as distinct species by the IUCN and published on the Red List. Gillaroo *S. stomachicus* and sonaghen *S. nigripinnis* were categorised on the Red List as 'Vulnerable' citing both species are a risk from eutrophication and introduced species. Ferox trout were also added to the IUCN Red List as *S. ferox* interestingly, however

they were categorised as 'Data deficient' based on a lack of published information the extinction risk of ferox trout could not be assessed (Freyhof & Kottelat, 2008).

1.9. THESIS OUTLINE

The main focus of this thesis is to investigate numerous aspects of the ferox trout life history. First, I examine the distribution and environmental characteristics associated with ferox trout populations in Scotland using robust statistical analysis. Secondly, I test the conventional models of ferox trout growth by comparing growth rates of sympatric ferox and brown trout in Scottish lakes. The successful capture of spawning ferox trout has not been achieved before, thus the third major aim of this thesis is to rear sympatric ferox trout and brown trout from eggs under common garden conditions and then subject the fish to a number of experimental manipulations and techniques to address fundamental questions and examine differences in physiology, behaviour and phenotype through ontogeny which may further our understanding on the potential mechanisms underlying ferox trout production. Finally, I discuss the within lake movements of sympatric *S. trutta* exhibiting alternative trophic phenotypes by examining their spatial distribution using acoustic telemetry.

Chapter 2 examines environmental parameters commonly associated with known ferox trout locations in Scottish lakes. I test (1) the significance of abiotic characteristics such as altitude (m.a.s.l), maximum lake depth (m), lake catchment area (km^2), lake surface area (km^2), and (2) a biotic factor, the presence of *S. alplinus*, on ferox trout distribution in Scotland. Based on results I produce a modern estimate on ferox trout distribution in Scotland.

Chapter 3 discusses the conventional models of ferox trout growth proposed by Campbell (1979) by examining the growth rates of sympatric populations of ferox trout and *S. trutta* from three Scottish lakes. I also examine the age structure of the ferox trout and sympatric *S. trutta* from these lakes. I test (1) whether ferox trout follow the conventional growth trajectory of slow growth followed by rapid growth after a switch to piscivory first proposed by Campbell (1979) and (2) if ferox trout have measureable differences in longevity across multiple locations.

Chapter 4 discusses differences in egg size, yolk sac size, hybrid viability, developmental rate, survival rate, SMR, MMR, AS, head shape morphology and lipid deposition between offspring from ferox trout parents and sympatric *S. trutta* parents held under common garden conditions. I test the effects of parental life history on (1) egg size and number; (2) yolk sac size of offspring; (3) early development and metabolic rate of offspring; (4) survival rate of offspring and the viability of hybrids; (5) offspring head

morphology and (6) lipid deposition in offspring. The results allow discussion of important mechanisms underlying the expression of ferox trout which may play a significant role in the production and continued maintenance of wild ferox populations.

Chapter 5 tests the first part of the hypotheses proposed by Kennedy & Fitzmaurice in 1971: 'are they naturally aggressive individuals which become predatory while still young?'. I discuss relative dominance of offspring from alternative life history parentage when competing for feeding territories. Using offspring from ferox trout and sympatric *S. trutta* parents reared on identical food rations and size corrected, I test (1) whether offspring from ferox trout and *S. trutta* parents differ significantly in colouration, aggression, feeding ability and spatial position in a semi-natural environment.

Chapter 6 discusses the spatial behaviour of two sympatric *S. trutta* ecotypes exhibiting alternate head shape morphology inhabiting a post-glacial lake. I test for (1) differences in head shape morphology using geometric morphometric analysis; (2) differences in home range size through comparison of Minimum Convex Polygons (MCP_{100}) and kernel utlisation distribution (KUD_{95}, home range) and (KUD_{50}, core range) using acoustic telemetry techniques; (3) differences in overall movement and (4) diurnal movement between ecotypes across a three-month period using acoustic telemetry.

Finally, Chapter 7 contains a general discussion and synopsis of the findings of this thesis, the contribution to our understanding of ferox trout, the limitations of these studies and the future direction of ferox trout research based on these findings.

CHAPTER 2.

LAKE BATHYMETRY AND SPECIES OCCURENCE PREDICT THE DISTRIBUTION OF A LACUSTRINE FISH APEX PREDATOR

*Note: A version of this chapter has been published as a manuscript in the Journal of Fish Biology, Volume 88, Issue 4, Page 1648 – 1654.

2.1. ABSTRACT

This study examined the abiotic and biotic characteristics of ecosystems that allow expression of a life history called ferox trout, the colloquial name given to brown trout *Salmo trutta* adopting a piscivorous life history strategy, an apex predator in post-glacial lakes in northern Europe. One hundred and ninety-two lakes in Scotland show evidence of currently, or historically, supporting ferox *S. trutta*; their presence was predicted in logistic models by larger and deeper lakes with a large catchment that also support populations of Arctic charr *Salvelinus alpinus*.

2.2. INTRODUCTION

Apex predators are often associated with physically large habitat sizes, home ranges and generally low population densities (Ordiz *et al*., 2013). Recently, apex predators have also been shown to play a disproportionate role in the dynamics of their communities. They structure communities through top-down regulation effects (Estes *et al*., 2011), have been shown to increase community stability (Ripple & Beschta, 2007; Estes *et al*., 2011), promote higher community diversity (Crooks & Soulé, 1999; Beschta & Ripple, 2014) and maintain natural community trophic cascades (Power *et al*., 1985; Estes & Duggins, 1995).

Apex predator populations, however, are also highly vulnerable to decline and extirpation (Lindsey *et al*., 2007; Inskip & Zimmermann, 2009; Ordiz *et al*., 2013). They are, by definition, highly dependent upon populations at lower trophic levels and therefore vulnerable to any change in prey population size (Ford *et al*., 2010; Estes *et al*., 2011). Because of their trophic position, apex predators also form relatively small populations, thereby making them vulnerable to exploitation, in-breeding and genetic drift, and environmentally driven, density-independent regulation effects on the population (Friedlander & DeMartini, 2002; Schindler *et al*., 2002; Fontaine *et al*., 2012). Unsurprisingly, the dynamic interactions of habitat and biotic communities that support and maintain apex predator communities is relatively poorly understood (Madin *et al*., 2010) and more acute for apex predators in aquatic environments (Ruttenberg *et al*., 2011).

The brown trout *Salmo trutta* L. 1758 is a highly polyphenic fish species, expressing a wide range of phenotypes (Ferguson, 1989; Jonsson, 1989; Klemetsen *et al*., 2003). In some places it can adopt a life history pattern associated with specialism for foraging on other fishes (Ferguson & Mason, 1981; Duguid *et al*., 2006). These so-called ferox trout *S. trutta* are piscivorous, generally grow to large size and are mostly, but not solely, lacustrine and long-lived (Campbell, 1971, 1979; Svalastog, 1991). This life-history type is not found in all systems where the species is present (Campbell, 1979), but where it is, it can be classified as an apex predator. In Scotland, the ferox life-history type has been described in 58 locations (Campbell, 1979) from the many thousands of sites that support *S. trutta*, but this is probably an underestimate (Campbell, 1979; Greer, 1995; Duguid *et al*., 2006). Identifying the mechanisms that promote expression of the life history type is a fundamental question in ecology; identifying lakes which support ferox *S. trutta* is essential to help direct conservation strategies.

2.3. Materials And Methods

This study collates historical data on the distribution of lacustrine piscivorous *S. trutta* in Scotland from a wide range of sources. It combines distribution with data on lake characteristics to predict the underlying environmental characteristics that promote the emergence and maintenance of this life-history form in Scotland.

To collate published records of ferox *S. trutta*, 20 angling books containing information on *S. trutta* locations in Scotland were digitized and scanned for records of: ferox *S. trutta* and large brown trout *S. trutta*. Online resources were also scanned using the same criteria. Each fish record associated with an estimated mass in excess of $2 \cdot 3$ kg (5 lbs) was deemed to be a ferox record. At this size it is generally accepted a fish must exhibit some form of piscivory and therefore may be considered to be exhibiting a ferox life-history pattern (Campbell, 1979).

The relationship between ferox *S. trutta* likelihood (presence or absence) and abiotic and biotic lake characteristics, was tested in logistic models. Predictor (independent) variables included; lake altitude (m.a.s.l), lake catchment area (km^2), lake area (km^2), maximum lake depth (m), mean lake depth (m) and the presence of Arctic charr *Salvelinus alpinus* (L. 1758). Bathymetric data for lakes (n = 610) were provided by Murray & Pullar (1910). Irrespective of age, these surveys remain the most authoritative in Scotland (Walker *et al*., 1988; Farmer *et al*., 1997; Pugh *et al*., 2011; Dudley *et al*., 2012; May & Spears, 2012). The presence of *S. alpinus* was provided by P. S. Maitland & C. E. Adams (unpublished data). Final model selection was determined by analysis of variance (ANOVA)

and selection of the lowest AIC rank (Zuur *et al*., 2009). The fit of the final model was tested by calculating the explained deviance (D^2), calculated as [null deviance – residual deviance (null deviance) −1], corrected by the number of *d.f.* used to fit the model (adjusted-D^2), equivalent to the R^2 of a linear least-squares regression model (Guisan *et al*., 1999; Leyk & Zimmermann, 2004). The predictive accuracy of the final model was tested by using a one-out cross-validation and 10-fold cross-validation (Elith *et al*., 2006). The magnitude of multi-collinearity between predictor variables was tested using a variance inflation factor (V_{IF}) test. All logistical analysis were conducted using R statistical a variance software inflation 3.1.2 factor (R Core Team; r-project.org/).

To generate a logistic probability model, an additional separate one-predictor model was fitted to extract coefficients for ferox *S. trutta* presence and lake area (km^2) alone. A probability model using these coefficients was constructed. The resulting probability of ferox *S. trutta* presence (%) was multiplied by the number of lakes (n=3788) found at a given area (km^2), provided by Smith & Lyle (1979).

2.4. RESULTS

Following an extensive review of the literature, this study found records from 192 lakes that currently support, or have historically supported a ferox *S. trutta* population in Scotland. A high V_{IF} between maximum and mean depth was found (V_{IF} >10), maximum depth was subsequently removed from analysis and mean depth retained. All independent variables were significant predictors of ferox *S. trutta* presence ($P < 0 \cdot 05$) except lake altitude ($P > 0 \cdot 05$). A significant interaction between *S. alpinus* presence and lake area was also discovered ($P < 0 \cdot 05$), the presence of *S. alpinus* increased the likelihood of ferox *S. trutta* in medium to larger sized lakes (Fig. 2.1.). The final model accounted for $0 \cdot 37$ (adjusted-D^2) of deviance explained and the predictive power of the final model was found to be 88% (Table 2.1). The presence of ferox *S. trutta* populations is also strongly associated with environmental factors (Table 2.2.). Probability models estimate that at least 366 lakes in Scotland have a surface area large enough to theoretically support a ferox *S. trutta* population (Table 2.3.). This is a comparatively small percentage ($9 \cdot 7$%) of sites, given the thousands of sites where *S. trutta* occur in Scottish lakes.

Table 2.1. Final selected model variables; Degrees of freedom (*df*), percentage of deviance explained by final model (adjusted-D^2), one-out and ten-fold cross-validation predictive error (CV) and final model AIC.

df	Adjusted- D^2	CV (One out)	CV (10-fold)	AIC
5,609	0.37	0.12	0.12	363.4

Table 2.2. Final logistic model containing environmental parameters associated with known ferox trout lakes in Scotland. All independent variables significant in predicting the presence of ferox trout population (highlighted in bold).

Characteristic	Estimate	Std.Error	z-value	*p*
Mean Depth	0.04	0.02	2.15	**0.032**
Area	0.01	0.01	4.76	**<0.001**
Catch	0.01	0.01	3.97	**<0.001**
Charr	1.72	0.39	4.39	**<0.001**
Area*Charr	-0.01	0.01	-3.13	**<0.01**

Table 2.3. Estimation of lakes which could theoretically support ferox trout based on lake area alone. Probabilities generated from logistic model coefficients. Data for lake area provided by Smith & Lyle (1979).

Km^2	Number of Lakes	Probability (%)	Estimate of ferox lakes
0.125	2973	7	208.11
0.38	417	9	37.53
0.75	209	12	25.08
1.5	83	21	17.43
3	54	51	27.54
6	27	94	25.38
12	12	99	11.88
24	**9**	100	9
51.5	4	100	4
Total	3788		365.95

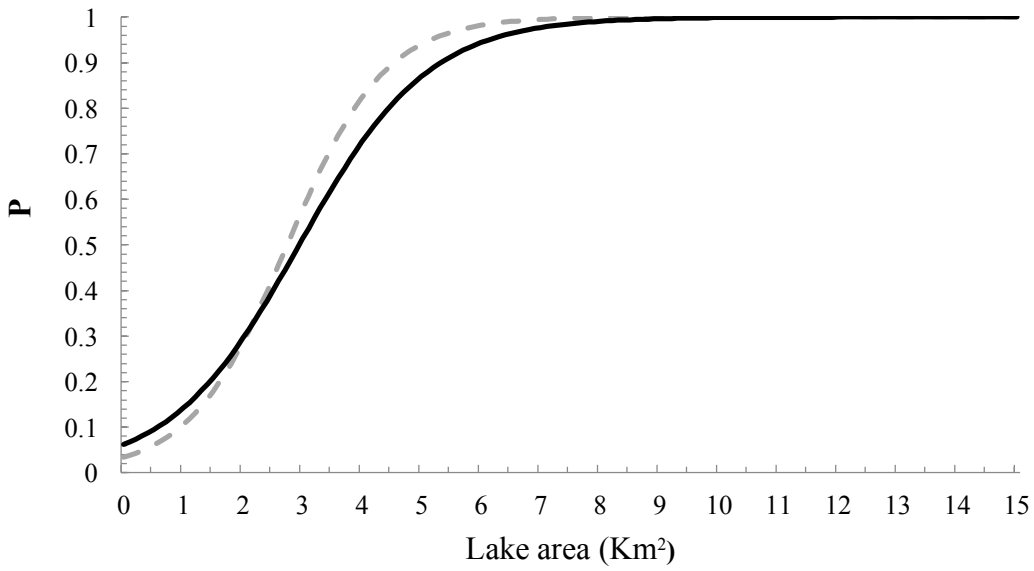


Figure 2.1. Probability (P) of ferox trout occurrence at lake area (km^2). Lake area without *S. alpinus* (solid black line), lake area with *S. alpinus* (dotted line).

2.5. DISCUSSION

The limited distribution of ferox *S. trutta* and their apparent low density in lakes that they do inhabit (Duguid *et al*., 2006) suggests a level of vulnerability of populations. The association between *S. alpinus* and ferox *S. trutta* suggested by Campbell (1979) was tested and is well-supported by the data ($P < 0 \cdot 001$) presented here. Given the variable nature of aquatic ecosystems, it is difficult to generalise, but it is highly likely that *S. alpinus* provide a prey source (Greer, 1995; Grey *et al*., 2002) that is driving expression of this life-history type. Other strong relationships between ferox *S. trutta* and their environment were also discovered. Lake depth ($P < 0 \cdot 05$), lake area ($P < 0 \cdot 001$) and lake catchment area ($P < 0 \cdot 001$) were all positively correlated with the presence of ferox *S. trutta*. Increased lake habitat heterogeneity through size and depth is positively correlated with an increase in species diversity (Eadie & Keast, 1984; Benson & Magnuson, 1992; Post *et al*., 2000). The low probability of ferox *S. trutta* occurrence in small lakes may be the result of low prey availability. Given piscivorous *S. trutta* have higher energy demands than insectivorous *S. trutta* and are feeding at the top of the food chain (Elliott & Persson, 1978; Elliott & Hurley, 2000; Jensen *et al*., 2006), small lakes may fail to meet the daily energy requirements necessary to support ferox *S. trutta*. Therefore, habitat size, the presence of a suitable prey species and the abundance of prey appear to be essential to maintain the piscivory expressed in ferox *S. trutta*.

Ferox *S. trutta* are comparatively large animals in post-glacial lakes. They require large habitats and the presence of a prey species, and where they do exist, distribution and population densities appear to be low (Ferguson & Mason, 1981; Greer, 1995; Grey *et al*., 2002).

In view of the wealth of literature illustrating the conservation value of apex predators in ecosystems (Power *et al*., 1985; Estes & Duggins, 1995; Crooks & Soulé, 1999; Ripple & Beschta, 2007; Estes *et al*., 2011; Beschta & Ripple, 2014), it is highly likely that ferox *S. trutta* play an extremely important role in the continued maintenance of top down processes in post-glacial lakes where they occur. Their rarity implies a level of vulnerability to environmental change and exploitation. Further studies on fine-scale ecological and biological mechanisms associated with ferox *S. trutta* will aid the design of appropriate management strategies for future implementation.

CHAPTER 3.

CONTRASTING GROWTH TRAJECTORIES BETWEEN SYMPATRIC *S. trutta* EXHIBITING ALTERNATIVE LIFE HISTORY STRATEGIES

*Note: A version of this chapter has been submitted as a manuscript to the journal, Ecology of Freshwater Fish.

3.1. ABSTRACT

Large piscivorous and long lived brown trout *Salmo trutta*, colloquially known as ferox trout, have been described from a number of oligotrophic lakes throughout Britain and Ireland. The 'ferox' life history strategy is associated with accelerated growth potential and extended longevity, (oldest recorded in the UK – 23 years old) thus ferox trout often reach much larger sizes and older ages than sympatric benthivorous trout. Conventional models suggest *S. trutta* adopting this life history strategy grow slowly before a size threshold is reached, after which, this gape limited predator switches diet to a highly nutritional prey source (fish) resulting in a measurable growth acceleration. This conventional model of ferox trout growth was tested by comparing growth trajectories and age structure of ferox trout and sympatric benthivorous trout in multiple lake systems in Scotland. In two of the three lakes examined sympatric trout life history strategies, exhibited distinct growth trajectories. In the third lake, a common pattern of growth was observed between life histories. Piscivorous trout were significantly older than sympatric benthivorous trout in all sites but ultimate body size was greater in only two of three sites. This study demonstrates that there are multiple ontogenetic growth pathways to achieving piscivory in *S. trutta* and, that the adoption of a piscivorous diet may be a major factor contributing to extension of life span in *S. trutta*.

3.2. INTRODUCTION

The brown trout *Salmo trutta* L. is a polytypic species that adopts a multitude of life-history strategies (Klemetsen *et al*., 2003). Lacustrine *S. trutta* frequently manifest as alternative life history strategies in sympatry, which often differ in colour (Ferguson & Mason, 1981), body size (Campbell, 1979), growth rate (Jonsson *et al*., 1999), feeding strategy (Grey, 2001) and longevity (Mangel & Abrahams, 2001). The rare piscivorous trout, colloquially referred to as ferox trout, occurs in a number of lacustrine systems from County Kerry in Ireland to the Ural Mountains in Russia and is found throughout lakes of Northern Britain and Ireland (Greer, 1995). Ferox trout are known to grow much larger and live longer

than sympatric lacustrine brown trout that feed on macrobenthic invertebrates throughout their life (hereafter referred to as benthivorous trout) (Campbell, 1979; Greer, 1995; Mangel, 1996; Mangel & Abrahams, 2001).

Both environmental and genetic factors have been implicated in the expression and maintenance of the ferox life history (Campbell, 1979; Ferguson & Mason, 1981; Duguid *et al*., 2006; McKeown *et al*., 2010). While the occurrence of ferox trout is strongly correlated with specific lake environment conditions (Campbell, 1979; Greer, 1995; Hughes *et al*., 2016a), in Lough Melvin (Ireland) and both in Loch Awe and Loch Laggan (Scotland), ferox trout are reproductively isolated and genetically distinct from sympatric benthivorous trout (Ferguson & Mason, 1981; Ferguson & Taggart, 1991; Prodöhl *et al*., 1992; Duguid *et al*., 2006).

Based on scale samples, and using the back-calculation approach, Campbell (1979) investigated growth rate patterns in ferox trout. This proposed that *S. trutta* adopt piscivory, after a period of relatively slow growth. However, once a size threshold is attained (Campbell indicated this being around 30cm) this gape limited predator is able to access fish prey, and this triggers a period of rapid growth occurs (i.e. as a direct result of the switch to a highly nutritional prey Campbell, 1979). In contrast, L'Abée Lund *et al*., (1992) concluded from stomach content analysis of *S. trutta* ranging from 11cm – 50cm in length, a diet switch to piscivory at a much smaller size (13cm). This suggests that the size at which piscivory is adopted is variable across populations and opportunistic, depending on the availability of prey of the right size (i.e. that the switch to piscivory is a least partly environmentally dependent). The diet switch in ferox trout has been linked to an extended life span (Campbell, 1979; Mangel & Abrahams, 2001). This contradicts a commonly cited concept that caloric restriction extends lifespan (Mangel & Abrahams, 2001).

Evidence from the literature (McKeown *et al*., 2010), suggest that, at least in some locations, when populations of piscivorous ferox trout and benthivorous trout occur in sympatry, these alternative life histories may not have originated from a single, common, post glaciation invading ancestor. Instead, they have resulted from multiple colonisation events of different ancestors. For example, ferox trout in Loch Awe and Loch Laggan (Scotland), were found to be genetically more closely related to ferox trout in Lough Melvin (Ireland), than benthivorous trout in the same lake (Duguid *et al*., 2006). Ferox trout are characterised by a high frequency of the lactate dehydrogenase 100 (*LDH-C1*100*) allele, as opposed to the alternative *LDH-C1*90* allele, which is more frequent in benthivorous brown trout (Hamilton *et al*., 1989; McMeel *et al*., 2001). Therefore at least in Lough Melvin, Loch Awe and Loch Laggan, ferox trout belong to a different ancestral genetically

defined lineage in relation to the sympatric benthivorous trout (Ferguson & Taggart, 1991; Duguid *et al*., 2006).

The alternative views on the taxonomic classification of ferox trout as either a genetically distinct species, or an adopted life history strategy of the *S. trutta* species complex is still subject of some debate, particularly around the lack of genetic support for alternative views. Recent review of the taxonomy status of ferox trout by Freyhof & Kottelat (2008), however, argues for a reinstatement of the full species status as suggested by Ferguson (2004). This view is also supported by McKeown *et al*., (2010) who carried out a comprehensive phylogeographic study of *S. trutta* in Britain and Ireland with focus on the origin(s) of the Lough Melvin *S. trutta*. The full species status of ferox trout is currently used by the IUCN, where the species is described as *Salmo ferox* Jardine, 1835, and it is listed in the category of "Data Deficient" on the red list of threatened species.

The aim of the study presented here was to examine some of the early ontogenetic processes in sympatric benthivorous trout and ferox trout, and specifically to test if the conventional model of ferox trout growth (slow growth followed by a growth acceleration after adoption of piscivory) is the common pattern across multiple ferox trout populations.

Thus three hypotheses are tested here:

1. During very early ontogeny, trout adopting a piscivorous or a benthivorous life history both grow at approximately the same rate.
2. That following a switch to piscivory, piscivores grow faster than benthivorous trout.
3. Following a switch to piscivory, piscivores exhibit extended life spans.

3.3. Materials And Methods

Three oligotrophic freshwater lakes in Scotland where ferox trout is known to occur in sympatry with benthivorous trout were sampled: Loch Awe in west-central Scotland, which drains to the west (56^{o} 55' N; 4^{o}25' W), Loch Rannoch in central Scotland, which drains to the east (56^{o} 40' N; 4^{o}18' W) and Loch na Sealga in northern Scotland, which drains westward (57^{o} 47' N; 5^{o}18' W).

Salmo trutta were sampled over a number of years (1997 – 2015) from Loch Awe (n=72) and Loch Rannoch (n=111) using Nordic gill nets or a non-destructive, specialised rod and line trolling technique used by experienced anglers (Thorne *et al*., 2016). All *S. trutta* from Loch na Sealga (n=37) were collected using Nordic gill nets, each comprising 12 mesh sizes ranging from 5 to 50mm (Appelberg *et al*., 1995). The cryptic nature of ferox trout and the difficulty of capturing sufficient number of specimens is well known, thus the sample

size used in this study is relatively large compared with previous studies and was only achieved by multiple year sampling (Duguid *et al*., 2006).

S. trutta were classified as piscivorous (and thus ferox trout) on the basis of morphological criteria as described by Campbell (1979) and Cawdery & Ferguson (1988). Thus, ferox were defined as *S. trutta* expressing large heads and obvious teeth relative to the size of the body and by total body size. These features were found to vary according to sampled lake. Thus, in Loch Awe, *S. trutta* > 420mm fork length (FL), and in Loch Rannoch, *S. trutta* > 400mm FL were classified as ferox trout. Using the same criteria, *S. trutta* were classified as benthivorous in Loch Awe if ≤ 360mm FL, and in Loch Rannoch if ≤ 310mm FL. This classification criterion has been previously validated for the identification of both piscivorous and benthivorous *S. trutta* using stable isotope analysis in Scottish lakes (Grey, 2001; Hughes *et al*., 2016b).

S. trutta from Loch na Sealga were killed on collection for a separate population study, and were classified as piscivorous or benthivorous based on stomach content analysis. Stomach contents that comprised exclusively fish were classified as piscivorous, and those that contained exclusively macroinvertebrates classified as benthivorous. Samples sizes of piscivorous trout were as follows: Loch Awe N=33, Loch Rannoch N=71 and Loch na Selaga N=14 and benthivorous trout: Loch Awe N=39, Loch Rannoch N=40 and Loch na Sealga N=23 (Table 3.1.).

Muscle tissue samples were taken from all trout from Loch na Selaga and stored in ethanol until analysis. Genomic DNA was extracted from biopsy tissue samples using the Promega DNeasy 96 kit, following the manufacturer's instructions. Each fish was subsequently screened for the presence of the ferox linked *LDH-C1*100* allele following protocol described by McMeel *et al*., (2001).

Scale samples were taken from above the lateral line of each fish from all sites and stored in paper scale packets. Scale samples were pressed onto acetate using a jeweller's press (DRM 150 press). Imprinted acetates were viewed using a Projectina scale reader. Scales were read following guidelines by Shearer (1992) and definitions from Berg & Grimaldi (1967) and life history terminology used by Allen & Ritter (1977).

For length-at-age estimates, measurements were made from the scale focus along the longest axis to the edge of the scale (*St*), and to the annulus being examined (*Sf*). Thus, the length of the fish at the time a feature was laid down (*LF*) was estimated by:

$$LF = Lt\ (Sf / St)$$

LF = back-calculated fish length at annulus *f*;

Lt = fish fork length at capture;

Sf = scale length to annulus *f*;

St = total scale length *t*.

Von Bertalanffy growth curves were constructed for each population but only for the first six years of growth; the rationale for this being that no benthivorous trout sampled were found older than this age. Population specific Von Bertalanffy growth curves were compared using Likelihood Ratio Tests in R statistical computing and graphics software using the *fishmethods* package (R Core team, 2016).

$L(t) = Linf*(1 - exp(-K*(t-t_0)))$

L(t) = Von Bertalanffy growth curve for size (t);

Linf = asymptotic length where growth is zero;

K= growth rate;

t_0 = theoretical age at size zero.

Since age structure data were not normally distributed, the non-parametric Wilcoxon signed-rank test was used to test for statistical significance for age at capture between ferox trout and benthivorous trout in each lake.

3.4. RESULTS

The population specific modelled Von Bertalanffy growth rate (K) determined over the first six years of growth, differed significantly between ferox trout and benthivorous trout in Loch Rannoch and Loch Awe (P <0.001) (Table 3.1. & Table 3.2.). There was no significant difference, however, in growth rate between these alternate life history strategies over this period in Loch na Sealga (P > 0.5) (see Table 3.2.). The growth pattern of the two life history strategies differed substantively in nature between lakes (Fig 3.1.). Thus, in Loch Awe, the rate of individual growth from both age 1 to age 3, and from age 4 to age 6 was higher in ferox trout in comparison to benthivorous brown trout (Table 3.3). In contrast, in Loch Rannoch, there was no significant difference in growth rate between life history strategies for the first 3 years; however, ferox trout showed a higher growth rate from age 4 to age 6. In Loch na Sealga there were no significant differences in growth rate between life history strategies both from age 1 to age 3 and from age 4 to age 6 (Table 3.3.).

Age structure differed significantly in all three lakes, with ferox trout being significantly older in each lake: Awe ((mean age ± SE) benthivorous trout 3.4 ± 0.18 years; ferox trout 8.6 ± 0.36 years, P < 0.01); Rannoch ((mean age ± SE) benthivorous trout 4.8 ±

0.14 years; ferox trout 12 ± 0.29 years, $P < 0.01$); na Sealga ((mean age ± SE) benthivorous trout 3.8 ± 0.8 years; ferox trout 4.7 ± 1.4 years, $P = <0.01$) (Fig 3.1.).

No significant differences were observed in the frequency of the ferox lineage linked *LDH-C1*100* allele (exact probability test $P > 0.05$) between trout representing both life history strategies in Loch na Selaga.

Table 3.1. Von Bertalanffy growth parameters for the first 6 years of growth of each life history type in each study lake.

Location	Life History	K	Linf	t0	*n*
Loch Awe	Benthivorous trout	0.10	47	-0.06	39
	Ferox trout	0.13	100	0.13	33
Loch Rannoch	Benthivorous trout	0.27	34	0.31	40
	Ferox trout	0.13	62	0.25	71
Loch na Sealga	Benthivorous trout	0.30	34	0.22	23
	Ferox trout	0.31	33	0.38	14

Table 3.2. Likelihood ratios tests of growth parameters for the first 6 years of growth between sympatric ferox and benthivorous trout. Significant differences (P<0.05) are highlighted in bold.

Location	Parameter	Chi sq	df	*P*
Loch Awe	Linf	14.49	1	**0.001**
	K	11.69	1	**0.001**
	t_0	3.76	1	**0.052**
Loch Rannoch	Linf	31.48	1	**0.001**
	K	24.37	1	**0.001**
	t_0	1.66	1	0.198
Loch na Sealga	Linf	1.16	1	0.281
	K	0.41	1	0.522
	t_0	5.97	1	**0.015**

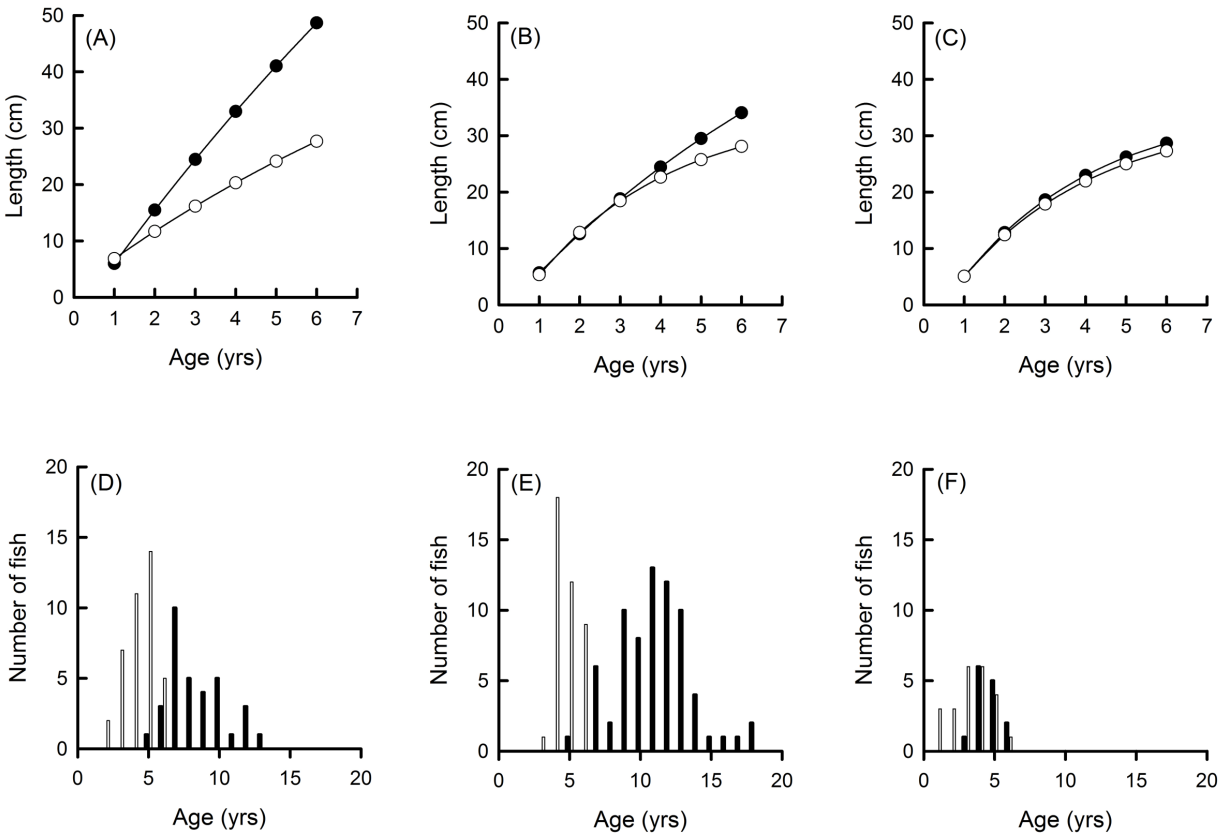


Figure. 3.1. Von Bertalanffy growth curves, on the first 6 years of growth, for ferox trout (●) and benthivorous trout (○) from each study lake; (A) Loch Awe, (B) Loch Rannoch, (C) Loch na Sealga and the age structure of ferox trout (■) and benthivorous trout (□) from each study lake; (D) Loch Awe, (E) Loch Rannoch, (F) Loch na Sealga.

Table 3.3. Comparison of length-at-age, using Welch's t-tests, in the first three years of growth and the last 3 years of growth between ferox trout and benthivorous trout from the same lake system. Significant differences are highlighted in bold.

Lake	Age	*t*	*df*	*P*
Awe	1 - 3 years	-6.32	146.08	**< 0.001**
	4 - 6 years	-14.78	130.77	**< 0.001**
Rannoch	1 - 3 years	0.88	236.09	0.38
	4 - 6 years	-3.05	252.37	**< 0.01**
na Sealga	1 - 3 years	-0.32	81.55	0.75
	4 - 6 years	-0.87	36.89	0.39

3.5. DISCUSSION

As gape limited predators *Salmo trutta* can only access prey items of a size which is relative to their body size (Steingrímsson *et al*., 2002; Jensen *et al*., 2008). Thus, *S. trutta* are not able to consume fish prey until they have a body size large enough to allow them to do so. A similar size threshold effect is known to operate in the closely related Arctic charr *Salvelinus alpinus* (Fraser *et al*., 1998). Since a single fish prey item provides higher levels of energy; growth rates are consistently faster among fish eating trout (Elliot & Hurley, 2000).

This increase in growth rate following a period of relatively slow growth is the conventional model of a ferox trout growth trajectory (Campbell, 1979), where the switch to a high nutritional prey source shows a measurable growth response after 'typical' brown trout growth. *S. trutta* in Loch Rannoch conform to this model, where trout exhibiting both life history strategies grow at approximately the same rate (hypothesis one) but ferox express higher growth following a switch to piscivory (hypothesis two) and show an extended life span (hypothesis three).

Piscivory in Loch na Sealga, however, contrasts distinctly with this model. Here, there is no accelerated growth effect of piscivory and, hence, hypothesis two is not supported. However, there is consistent evidence that ferox trout do live longer in all three lakes, supporting hypothesis three (Fig. 3.1). Contrary to observations elsewhere (Duguid *et al*. 2006), there was no evidence in this study for the presence of the *LDH-C1*100* allele in piscivorous *S. trutta* in Loch na Sealga.

Loch Awe trout also do not conform well to the conventional model of ferox growth. A high growth rate of ferox trout adopting a ferox life history strategy was noted at a very early age (ca between age 1 and age 2 and small size ca 100mm FL - Fig. 3.1 and Table 3.3). There are two possible explanations for the growth pattern observed in Loch Awe. First, ferox trout in Loch Awe may switch to piscivory at a very young age (ca 1+ years) and very small body size <100mm. This is very unlikely, as piscivory is not known to occur at this size in *S. trutta* elsewhere (Campbell, 1979; L'Abée Lund *et al*., 1992), however this explanation is potentially possible if fish prey were available at very small sizes (Juanes, 1994; Mittelbach & Persson, 1998). Alternatively, and more plausible, is that trout in Loch Awe, adopting a ferox trout life history comprise the fastest growing individuals before a switch to piscivory. This explanation does not support hypothesis one. The observed high growth rate in young ferox trout in Loch Awe suggests that, if this mechanism is in operation, they are highly efficient at acquiring food in a quantity and/or quality as juveniles that

exceeds that of trout that adopt a benthivorous life history strategy. This probability is supported by the evidence of higher dominance levels in ferox trout progeny elsewhere (Hughes *et al*., 2016b). This final assertion implies that there may be a level of inheritance of life history strategy present in the Loch Awe ferox population. This is likely, certainly for Loch Awe trout, where previous genetic studies found the ferox trout population is reproductively isolated from the sympatric benthivorous trout population (Duguid *et al*., 2006).

In Loch na Sealga, the study presented here demonstrates that the ferox life history strategy can be found in the absence of the *LDH-C1*100* allele. Thus it is most probable that ferox life history strategies predominate in populations with a high frequency of the *LDH-C1*100* allele but can still occur, possibly at a lower frequency in populations that do not exhibit the *LDH-C1*100* allele. It also shows that not all *S. trutta* that reach a size threshold that allows a switch to piscivory do actually make the switch, which is true of all lakes in this study, but most obvious in Loch na Sealga and Loch Rannoch. This may be due to constraints faced by apex predators, such as that of prey availability (Ford *et al*., 2009). At least in some, but not all locations (demonstrated by Loch Awe), it is very likely that ferox trout comprise the fastest growing individuals during very early ontogeny prior to switching to fish contradicting hypothesis one. Extraordinarily, there is a consistent pattern across lakes that suggests piscivory confers an extended life span on individuals adopting this life history strategy, the mechanisms through which this may manifest are understudied but worthy of considerable future attention.

The conclusion of this study is that there are multiple ontogenetic routes to reaching piscivory in *S. trutta*. In at least one of the lakes sampled, Loch Rannoch, ferox trout growth conformed to the conventional model of slow growth followed by fast growth after a switch to piscivory, however this was not evident in two other lakes sampled. These data demonstrate individuals exhibiting similar phenotypes as adults, may have employed entirely different strategies as juveniles.

CHAPTER 4.

DIFFERENCES IN EARLY ONTOGENY OF *S. TRUTTA* FROM ALTERNATIVE LIFE HISTORY PARENTAGE

4.1. ABSTRACT

Measuring traits that contribute to observed life-history strategies within a species is difficult. Salmonids provide a model species to examine both trophic polymorphisms and physiological characteristics under laboratory conditions. In this study I examined full sibling and half sibling hybrid offspring from two sympatric potamodromous trophic polymorphisms of brown trout *Salmo trutta*; the rare piscivorous ferox trout (which grows to a large size and has delayed maturity) and the common benthivorous brown trout (which grows to much smaller sizes but matures earlier). By rearing offspring from eggs of known parentage under common garden conditions, I examined key biological traits (egg size, yolk sac size, juvenile development, juvenile mortality, lipid deposition, metabolic rate and head morphology) which are likely to contribute to adopted life-history strategies. I found offspring from ferox trout parentage varied in some, but not all biological traits measured; compared to benthivorous brown trout, with hybrids often falling between the ranges. Ferox trout eggs and offspring yolk sacs at emergence were larger than benthivorous brown trout. Ferox trout offspring had higher survival rates, lower body lipid content and significantly different head shape morphology compared with benthivorous brown trout. I found no differences in metabolic rate or pace of embryonic development between offspring of ferox and benthivorous parents. In this chapter, I provide strong evidence that the life-history adopted by the parents, is likely to influence the life history trajectory of their offspring.

4.2. INTRODUCTION

Intraspecific variation can manifest as differences in genetic structuring, phenotypic expression and life history traits within a single population and is frequently observed within populations of fish inhabiting post-glacial lakes (Ward *et al*., 1994; Smith & Skúlason, 1996). Such diversity within a single population is believed to provide insights into the formation of new species (Schluter & Rambaut, 1996: Adams & Huntingford, 2002).

Genetic structuring in Atlantic salmon *Salmo salar* populations is well-documented (King *et al*., 2001; Primmer *et al*., 2006). Despite a large marine migration capability, there is surprisingly little gene flow between freshwater spawning tributaries, believed to be in part to their strong innate homing ability (Vähä *et al*., 2007). Thus, large intraspecific genetic

diversity exists within single *S. salar* populations, even in the absence of obvious physical barriers (Primmer *et al*., 2006; Dionne *et al*., 2008).

Intraspecific phenotypic variation is often, but not always, reflected in morphological differences between individuals exploiting alternate trophic niches. This is well-documented in three-spined sticklebacks *Gasterosteus* spp and Arctic Charr *Salvelinus alpinus* populations utilizing benthic and limnetic resources (Bentzen & McPhail, 1984; Schluter & McPhail, 1992; Klemetsen *et al*., 2003). For example, benthic sticklebacks possess short stout bodies, with wide mouths and few small gill rakers, shown to aid foraging ability on benthic invertebrates (Bentzen & McPhail, 1984; Schluter, 1993). In contrast, limnetic threespine sticklebacks have long bodies, pointed mouths and many long gill rakers, to aide filtration of small zooplankton from the water column (Bentzen & McPhail, 1984; Schluter, 1993; Peichel *et al*., 2001). In addition, benthic and limnetic polymorphisms of this species exhibit variation in body armor. Benthic sticklebacks have reduced or no dorsal spines, and a reduced number of lateral plates, compared with limnetic forms which retain dorsal spines and lateral plates (Reimchen, 1980). This variation in defense is thought to be a response to differences in predation pressure, linked to the distinct habitats each form occupies (Marchinko, 2009). For example, limnetic sticklebacks which operate in pelagic open waters are predated upon by birds and fish, thus large spines act as a useful deterrent (Vamosi & Schluter, 2004). Benthics which operate in near-shore areas are frequently predated by invertebrates (e.g. backswimmers, dragonfly larvae, diving beetles) consequently dorsal spines would be a less effective defense (Vamosi & Schluter, 2004).

Although phenotypic and genetic variation is fairly well described, there is a paucity of studies on intraspecific variation in life-history traits; as such traits are often cryptic and difficult to measure. Life history variation may have physiological (Smith & Fetwell, 1974; Van Leeuwen *et al*. 2011), ecological (Nussbaum & Schultz, 1989) or phylogenetic basis (Gotelli & Pyron, 1991).

Lacustrine Rainbow smelt *Osmerus mordax* commonly manifest as dwarf and 'normal' smelt life history strategies (Nellbring, 1989). Dwarf smelt share similar morphological features to limnetic ecotypes in other species but exhibit substantial variation in life history traits such as growth, length at age, age of maturity and reproductive strategy (Saint-Laurent *et al*., 2003). For example, dwarf smelt are semelparous (complete a single reproduction event before dying), grow to much smaller sizes and mature earlier, compared to the "normal" type smelt (Saint-Laurent *et al*., 2003) suggesting higher mortality in dwarf populations compared with normal populations (Saint-Laurent *et al*., 2003).

Brown trout *Salmo trutta*, are a highly polytypic species with high levels of genetic diversity, phenotypic plasticity and life history differentiation between populations. The rare piscivorous life history, known colloquially as ferox trout, has received little attention mainly due to the difficulty in acquiring adequate samples (Duguid *et al*., 2006), however ferox trout exhibit genetic, phenotypic and life history characteristics distinct from sympatric *S. trutta*. Ferox trout are described as dull brown or green with little or no spotting, with disproportionately long heads and wide mouths containing many large teeth (Ferguson & Mason, 1981; Ferguson & Taggart, 1991). Electrophoretic studies have found ferox trout to be genetically distinct and reproductively isolated from sympatric brown trout in some lakes in Ireland and Scotland (Ferguson & Mason, 1981; Ferguson & Taggart, 1991; Duguid *et al*., 2006). Remarkably, ferox trout exhibit extensive variation in life history traits. Ferox trout have been found to be consistently older and larger than sympatric *S. trutta* (Campbell, 1979; Mangel & Abrahams, 2001) and potentially have delayed maturation (Campbell, 1971; Duguid *et al*., 2006). Unlike physical adaptations to a specific trophic niche, the evolutionary benefit and mechanisms contributing to such variation in life history traits is unknown.

Thus, elucidating the source of variability observed in life-history strategies both among and within a species is complex and likely a result of both genetic and environmental influences (Chapman *et al*., 2011; Dodson *et al*. 2013). A conventional way to control external environmental conditions is through a common garden experiment. By raising organisms in identical rearing environments inherited variation and maternal effect influencing life history traits can be observed. To measure variation in life history traits, two life histories of the *Salmo trutta* species complex - the common benthivorous trout and the rare piscivorous ferox trout - were reared from eggs under common garden conditions.

Numerous traits considered important to life history development in salmonids were measured throughout early ontogeny including maternal effects (clutch size, egg size and yolk sac size of alevins), pace of embryonic development, juvenile survival rate, metabolism, lipid deposition and head shape morphology. The viability of ferox trout and benthivorous trout hybrids was also tested by creating female ferox trout x male benthivorous trout (FX:BT) and female benthivorous trout x male ferox trout (BT:FX) hybrids.

As ferox trout females are larger than benthivorous brown trout females I predicted ferox females would produce a greater quantity of larger eggs and thus ferox offspring would have larger yolk sacs and higher survival rates than offspring from female benthivorous trout. Given the high growth potential in adult ferox trout I also expected metabolic rate to be

higher in offspring of ferox trout. Equally, as ferox trout and benthivorous trout exploit different food resources, measurable differences in head shape morphology were expected.

4.3. Materials And Methods

4.3.1. Broodstock Collection

Broodstock (3 ferox females and 3 ferox males, 3 potamodromous brown trout females and 3 potamodromous brown trout males) were captured using fyke nets and electrofishing between 1 October and 12 November 2013 from two tributaries in the Loch Maree catchment, Scotland. Due to the rarity of ferox trout (Duguid *et al*., 2006), the likelihood of collecting ripe females during spawning time is low. At capture, fish were classified as ferox trout or benthivorous brown trout based on size: ferox trout (40-80cm) benthivorous brown trout (20-35cm) (Campbell, 1979) and prior knowledge from local angling groups about spawning locations. Classification of the two life history types was confirmed by subsequent stable isotope analysis of egg samples. Mature fish were transported to holding tanks on the Coulin Estate, Kinlochewe, Scotland where they were held in two large 2000 L tanks supplied with river water and assessed daily for ripeness. On the 14 November 2013, all fish were anaesthetized, blotted dry, and their eggs or sperm extruded by abdominal massage. Eggs were fertilized by randomly selected males of the same life history type to create two full sibling families of each life history. Hybrids were also created by fertilising eggs from a single female brown trout with sperm from a single male ferox trout and eggs from a single female ferox trout with sperm of a male brown trout, referred to as paternal and maternal hybrids hereafter.

Fertilised eggs were transported to the Scottish Centre for Ecology and the Natural Environment (SCENE), Loch Lomond, Scotland. Each family was reared separately in mesh baskets held in clear plastic tanks (50cm X 30cm X 15cm) in a temperature controlled environment chamber (temp $7 \pm 0.08^{o}C$) using water on a partial recirculation system. Water was pumped directly from Loch Lomond before being channelled through a free-standing filter unit and a single in-line UV sterilizer. Eggs were held in complete darkness until hatching. Eggs were examined daily, with any dead eggs recorded and carefully removed. Three ontogenetic stages and the development time taken to attain these stages were recognised. Developmental events included the presence of eye pigmentation, hatch and yolk absorption – eye pigmentation was recorded when 100% of the eggs from a group exhibited visible black eye pigmentation. Similarly, day of hatch was recorded when 100% of eggs hatched for each family. Full yolk absorption was recorded when no visible yolk sac was

present on all alevins. Each developmental stage was recorded in terms of degree days (DD) - calculated as the cumulated mean water temperature recorded per day. After hatching, alevins were raised under an ambient photoperiod. After complete yolk absorption a standard commercial salmon pellet was introduced (Biomar, Aarhus, Denmark). Fry were fed at a rate of approximately 3% body wt. day^{-1}. On 5 April 2014 fish were moved to larger 175 L circular flow tanks. At this stage, families were mixed to create two tanks of equal density (160 fish per tank) for each life history, due to the high mortality rate, paternal and maternal hybrids were held in lower densities.

4.3.2. Stable Isotope analysis

Previous stable isotope analysis on ferox trout from Loch Ness demonstrated ferox trout have a significantly elevated $\delta^{15}N$ signature compared to benthivorous brown trout feeding on zooplankton or macroinvertebrates (Grey, 2001; Grey *et al*., 2002). Stable isotope analysis was conducted to confirm the presumed different foraging strategies of the broodstock used in this study.

Eggs (n=4) from each family were taken during stripping (total n=16) and dried for 96 hours at 48°C in a drying oven. The dried tissue was ground to a fine powder using a pestle and mortar. Half of each sample was lipid extracted as follows: 15 mg of ground tissue was soaked in a 2:1 (by volume) chloroform: methanol solvent mixture. After 20 minutes, the sample was centrifuged (3000 rpm for 5 minutes), the supernatant discarded and the process was repeated until the solvent ran clear. The lipid extracted samples were then dried for a further 96 hours at 48°C in a drying oven. Non-lipid extracted and lipid extracted samples (n=32) were measured (0.7-0.9mg) into tin capsules (standard weight 5 x 3.5mm). Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope ratios were determined by continuous flow isotope ratio mass spectrometry (CF-IRMS), using a Costech ECS 4010 elemental analyser coupled to a ThermoFisher Scientific Delta XP-Plus IRMS at the NERC Life Sciences Mass Spectrometry Facility.

4.3.3. Egg Size And Yolk Sac Size

Photographs of eggs were taken 48 hours after fertilization using a Canon EOS 350D digital camera, fixed to a camera stand and illuminated with two blue lights mounted on either side of the camera stand to ensure quality images. Three days after hatching, 10 alevins were removed from each family. Alevins were placed on the left ventral side and photographed using the same procedure stated above. For each photograph, a reference scale was added. Digital images were uploaded to a computer and both egg size and yolk sac

surface area were measured using ImageJ software (Version 1.2t, developed by Wayne Rasband, National Institutes of Health, U.S.A.; Jardine & Litvak 2003).

4.3.4. Survival Rate

Mortalities were recorded across three early life stages (egg fertilization to hatch; hatch to swim-up; egg fertilization to swim-up) and percent survival to each stage was calculated. Percent survival was transformed to survival rate by square root arcsine transformation prior to analysis (Findlay *et al*., 2009).

4.3.5. Lipid Content

After 9 months of exogenous feeding, lipid content was measured on every individual using a Distell FM 692 fat meter. The Distell fat meter measures the water content of a sample. The fat meter was calibrated to the fat - water relationship specific to brown trout prior to taking measurements. Two measurements were taken along the body on both sides of the fish, the ultimate value being the average percent body fat of the four readings.

4.3.6. Metabolic Rate

At 9 months old, fish were removed from holding tanks (water temperature 13.3 ± 0.8 °C) and metabolic measurements conducted using the following procedures.

To ensure metabolic rates were not influenced by digestion, fish were not fed for at least 28 h prior to metabolic measurements, fish aquaria were also siphoned to remove excess food. This has been shown as an adequate amount of time for salmonids to evacuate guts and eliminate specific dynamic action (SDA) or increased energy demands associated with digestion (Cutts *et al*., 2002; Rosenfeld *et al*., 2015).

Metabolic rate was measured as the amount of oxygen consumed by an individual over a set period of time. Each fish (ferox n = 15; brown trout = 16) was placed in a glass respirometry chamber (8.0cm length, 3.4cm diameter, 0.104 L volume) for a 24 h period from approximately 10:00am – 10:00am. Each chamber was immersed in standing water bath held in a temperature controlled room, water temperature remained at 13.6 $\pm$ 0.5 °C throughout the entire experiment. To ensure maximum water oxygen saturation an air-stone was placed in a second water bath which fed directly into the first water bath housing respirometry chambers. To prevent light and movement disturbances each chamber was wrapped in dark material for the duration of measurements and no lights were used in the temperature control room (Cutts *et al*., 2002). To minimise gas exchange across permeable surfaces, glass respirometers and tygon tubing were used (Stevens, 1992).

Prior to introduction to respirometry chambers, each fish was placed in a 5 l bucket using a fine wire mesh hand net and chased until exhaustion (defined as the lack of capacity for burst swimming; Clark *et al*., 2012). Chase protocols are a common method for elucidating MMR in fishes (Soofiani & Priede, 1985; Reidy *et al*., 1995; Clark *et al*., 2013; Roche *et al*., 2013; Norin & Clark, 2016).

Fish were then introduced into a glass repirometry chambers and oxygen consumption was measured every 1s using an oxygen probe (Loligo systems, Tjele, Denmark) connected to an oxygen meter (FireStingO_2 oxygen meter; PyroScience). Measurement of oxygen consumption was achieved by creating a closed loop system within each chamber. A closed loop was created when water flow to each chamber was automatically turned off (Superpro MFRT-1 timer, Somerset, England) allowing for a decrease in oxygen to be measured. Water was circulated within this "off" period by the use of a peristaltic pump (Masterflex L/S, London, England). After a period of 20 min new oxygenated water was reintroduced, driven by a water pump (Eheim 300 universal, Deizisau, Germany) through each respirometry chamber for a further 25 min. This 20 min "off" and 25 min "on" cycle was repeated to ensure multiple oxygen consumption measurements were taken. To account for potential background oxygen consumption, chambers were cleaned after each 24 h measurement and the oxygen consumption within empty chambers was measured immediately before and after fish measurements were taken. This background oxygen consumption was subtracted from observed values for each fish.

The rate of oxygen consumption was determined using the following equation (Ege & Krogh, 1914):

$$MO_2=V_w(\Delta C_w0_2)/\Delta t$$

where V_w is the volume of water in the respirometer and associated tubing minus the volume of the fish and ΔC_w0_2 is the change in oxygen concentration of the water over time period Δt (Steffensen, 1989). Oxygen concentration was calculated by correcting PO2 (partial pressure oxygen) for barometric pressure and multiplying by αO2 (μmol L^{-1} $torr^{-1}$), the solubility coefficient at the observed temperature. Standard metabolic rate was estimated by using the average of the lowest 10% of values observed during the respirometry trial (Norin *et al*., 2014). Following respirometry measures all fish were anaesthetized, blotted dry and weighed to the nearest 0.0001g (Mass ± S.E.; Ferox 6.46 ± 0.46; brown trout 4.91 ± 0.48).

4.3.7. MORPHOMETRICS

Lateral view photographs of all fish were taken using a Canon EOS 350D digital camera fixed to a camera stand and illuminated with two blue lights mounted on either side of the camera stand to ensure quality images for geometric morphometric analysis. For each photograph, a scale reference was added to allow for the correction of shape change associated with changes in body size. Ten consistently identifiable landmarks were digitised on each fish image taken at 3 months, 6 months and 9 months, using tpsDig and tpsUtil software (Rohlf, 2006).

4.3.8. STATISTICAL ANALYSIS

To analyse trophic position of the broodstock identified using proximate characteristics of the two life history strategies, a Welch's t-tests was used to compare mean δ13C and δ15N derived from stable isotope analysis. Size differences in parental broodstock length were compared using general linear models (GLMs). To test for differences in egg and yolk sac surface area, a linear fixed effect model (LME) with family as a random effect was used to control for potential effect of family. To test for differences in clutch size, a Welch's t-test was used. Differences in offspring survival rate, metabolism and lipid deposition were analysed using ANOVA and post-hoc Tukey HSD tests. All statistical tests were all performed using R statistical software 3.1 (R Core Team 2014). The life history types and families are notated as follows: Ferox life history (FX) and bentivorous trout life history (BT). Families are described as female parent life history type: male parent life history type fish. For example:

Female ferox trout x male ferox trout (FX:FX) – ferox purebreds

Female brown trout x male ferox trout (BT:FX) – hybrid cross

Female ferox trout x male brown trout (FX:BT) – hybrid cross

Female brown trout x male brown trout (BT:BT) – brown trout purebreds

4.3.9. MORPHOMETRIC ANALYSES

To test for differences in head morphology, landmarks for each specimen at four stages of ontogeny (alevins, 3 months old, 6 months and 9 months old) were aligned, translated, rotated and scaled to a unit of centroid size using a Procrustes superimposition (Rohlf & Slice, 1990). To remove shape changes due to fish size, the Procrustes coordinates were regressed on log centroid size of the individual with the residual values used for all subsequent analysis. Discriminant function analysis (DFA) was undertaken in MorphoJ to assess the effect of life-history parentage on body shape, using the average Mahalanobis distance (D) between the two life-history groups from a single parent type (ferox or

benthivorous trout) for each time interval. To test for differences in shape change over time, discriminant function analysis coefficients were extracted for each fish and a linear regression model constructed for each life history strategy.

4.4. Results

4.4.1. Stable Isotope Analysis

Benthivorous trout eggs (n = 8) were significantly more depleted in $\delta^{15}N$ (t = -35.4, df = 13.1, $P < 0.01$) than ferox trout eggs (n = 8), due to benthivorous broodstock feeding at a lower trophic level than ferox trout broodstock. There was no significant difference in $\delta^{13}C$ (t = 1.4, df = 12.3 P = 0.2) between benthivorous trout and ferox trout eggs. Similarly, in lipid extracted eggs samples, benthivorous trout eggs (n = 8) were significantly more depleted in $\delta^{15}N$ (t = -35.2, df = 13.8, $P < 0.01$) than ferox trout eggs (n = 8) and there was no significant difference in $\delta^{13}C$ (t = 0.2, df = 12.8, P = 0.84), (Fig. 4.1.: Table 4.1. & 4.2.).

4.4.2. Broodstock Length

As expected, offspring from ferox trout were larger overall at time of spawning than benthivorous trout ($F_{3,8}$ = 214.87, $P < 0.001$). However, there was no statistically significant differences in length between female ferox trout and male ferox trout ($F_{1,4}$ = 5.62, P = 0.07), nor between female benthivorous trout or male benthivorous trout ($F_{1,4}$ = 0.03, P = 0.87).

4.4.3. Egg, Yolk And Clutch Size

Parental life history affected both egg (χ^2 = 4.1342, df = 1, P < 0.05) and alevin yolk sac size (χ^2 = 7.409, df = 1, P <0.01). Eggs from female ferox trout were larger (mean ± SE;31.19 mm^2 ± 0.152) than female benthivorous trout (27 .49mm^2 ± 0.24) and alevins from female ferox trout had on average a larger yolk sac surface area (36.80 mm^2 ± 0.85) than alevins from female benthivorous trout (28.95 mm^2 ± 0.48). However, there was no significant difference in clutch size between ferox trout and benthivorous trout (t = 1.45, df = 2.87, P =0.25) (Table 4.3.).

4.4.4. Embryonic Developmental Pace

There was no significant difference in developmental pace between life history strategies i.e. eye pigmentation, hatch date or swim-up (yolk absorption) (Table 4.4.).

4.4.5. Embryonic Survival Rate

Survival rate from fertilisation to hatch time differed significantly between parental life history type ($F_{2,3}$ = 1246, $P < 0.001$; Fig 4.3.). Post hoc tests revealed eggs from ferox trout (FX:FX) had significantly higher survival rates from egg to hatch than all other life

history types $P < 0.01$. Eggs from maternal ferox hybrids (FX:BT) and paternal ferox hybrids (BT:FX) had significantly higher survival rates from egg to hatch than brown trout (BT:BT) $P < 0.10$, but there was no difference in survival between the two hybrid families $P = 0.52$ (Fig. 4.3.).

Survival rate from fertilisation to swim-up also differed significantly depending on parent life history ($F_{2,3} = 528$, $P < 0.01$). A post hoc Tukey test revealed eggs from ferox trout (FX:FX) had a significantly higher survival rate than all other life history types $P < 0.05$ with paternal ferox offspring (BT:FX) having the lowest survival (Fig. 4.3.).

4.4.6. Metabolic Rate

There was no significant difference in standard metabolic rate (SMR), maximum metabolic rate (MMR) or aerobic scope between offspring from ferox trout (FX:FX) and brown trout (BT:BT), $P > 0.05$ (Fig. 4.4.).

4.4.7. Lipid Deposition

Percent (%) body lipid differed significantly as a function of parent life history type ($F_{3,821} = 11.02$, $P < 0.001$). A post hoc Tukey test revealed ferox trout offspring (FX:FX) had a significantly lower % body lipid than brown trout offspring (BT:BT), $P < 0.001$ and maternal ferox trout hybrids (FX:BT), $P = 0.049$. There was no significant difference in % body lipid between all other life history offspring (Fig 4.5.).

4.4.8. Head Morphology

There was a significant difference in head morphology between ferox trout offspring (FX:FX) and brown trout offspring (BT:BT) during 4 periods of development, (alevins: Mahalanobis distance D = 1.9790 $P < 0.001$ (Fig. 4.5.(A)); 3 months D=3.0445 $P < 0.001$ (Fig. 4.5.(B)); 6 months D = 2.3509 $P < 0.001$ (Fig. 4.5.(C)); 9 months D = 2.1224 $P < 0.001$(Fig. 4.5.(D))).

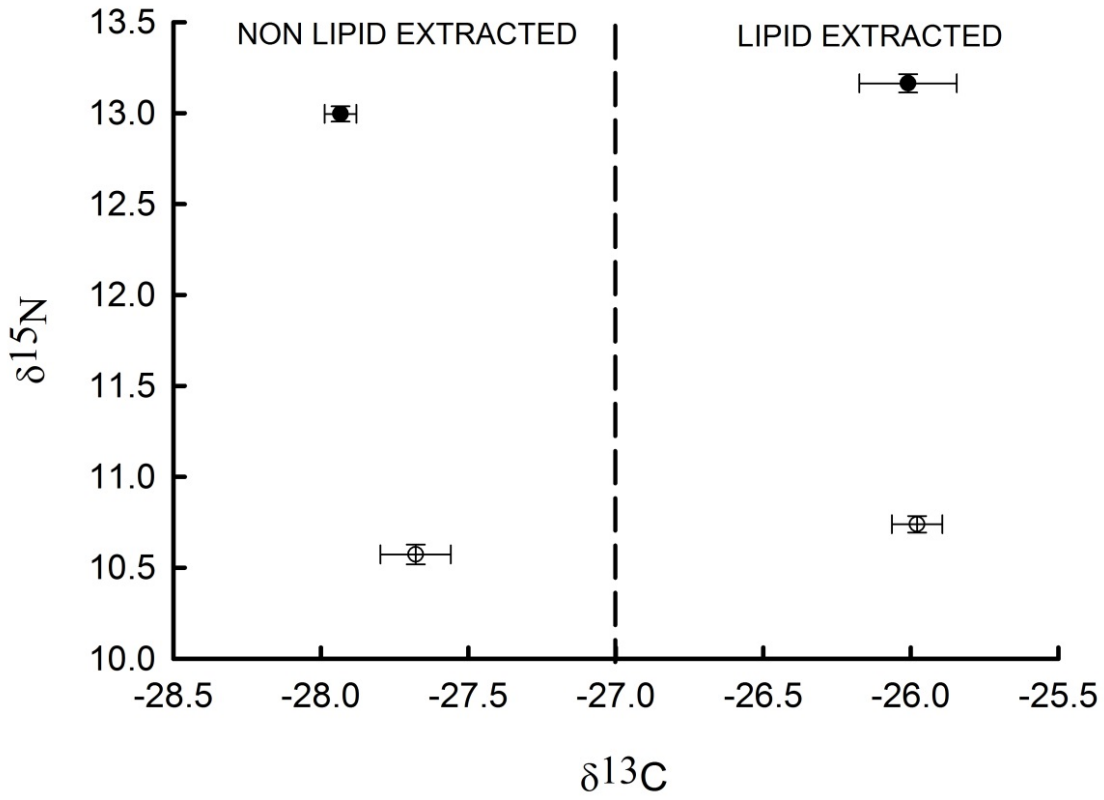


Figure. 4.1. Stable isotope ratios of carbon and nitrogen in brown trout (□) and ferox trout (■) from Loch Maree catchment.

Table 4.1. Result from 'non-lipid' removed stable isotope analysis of *δ* C13 and *δ* 15N from eggs from both life history forms.

Family	Morph	δC13(%)	δN15(%)
1	Ferox trout	-28.04 ± 0.06	12.9 ± 0.02
2	Ferox trout	-27.82 ± 0.05	13.09 ± 0.04
5	Brown trout	-27.68 ± 0.12	10.45 ± 0.03
6	Brown trout	-27.93 ± 0.06	10.7 ± 0.05

Table 4.2. Result from 'lipid' removed stable isotope analysis of δ C13 and δ 15N from eggs from both life history forms.

Family	Morph	δC13(%)	δN15(%)
1	Ferox trout	-26.2 ± 0.15	13.06 ± 0.07
2	Ferox trout	-25.8 ± 0.02	13.3 ± 0.01
5	Brown trout	-26.03 ± 0.02	10.7 ± 0.1
6	Brown trout	-25.93 ± 0.03	10.79 ± 0.14

Table 4.3. Egg number and egg surface area (mm^2 ± S.E) of each family of offspring. Families from maternal ferox trout (1,2,4) and maternal brown trout (3,5,6).

Family	Life History	Egg Number	Egg Area (mm^2) ± S.E.
1	FX	122	30.67 ± 0.17
2	FX	267	32.45 ± 0.18
4	FX	402	29.73 ± 0.18
3	BT	392	25.24 ± 0.19
5	BT	328	30.44 ± 0.19
6	BT	462	26.83 ± 0.21

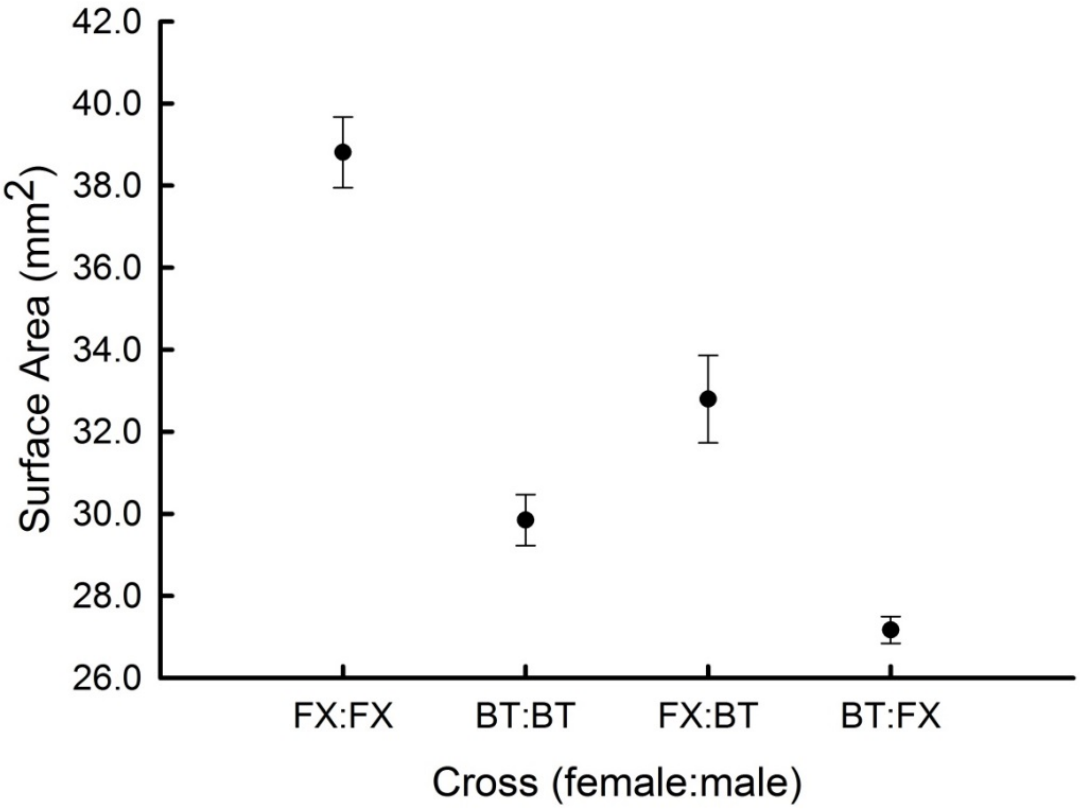


Fig. 4.2. Yolk sac surface area (mm^2) of 3 day old alevins, pure ferox trout (FX:FX), pure brown trout (BT:BT), maternal ferox trout and paternal brown trout hybrids (FX:BT) and maternal brown trout and paternal ferox trout hybrids (BT:FX).

Table 4.4. Key developmental stages during early development in degree days; eye pigmentation present in eggs, day of hatch and 'swim-up' stage or full yolk absorption stage.

Family	Life History	Eyed-egg stage	Hatch	Swim-Up
1	FX	239.1	492.9	852.2
2	FX	239.1	468.9	843.2
3	PC	246	508.4	877.8
4	MC	239.1	492.9	869.4
5	BT	227.5	484.8	869.4
6	BT	239.1	461	861

Table 4.5. Survival rates of each life-history to important developmental stages (Eggs to hatching; hatching to swim-up; Overall survival from egg to swim-up).

Family	Life History	Egg to Hatch	Hatch to Swim-up	Egg to Swim-up
1	FX	75.16	73.68	68.08
2	FX	75.38	73.15	67.84
3	PC	62.25	34.05	29.92
4	MC	62.97	62.15	51.49
5	BT	55.73	54.37	42.20
6	BT	56.33	56.40	43.88

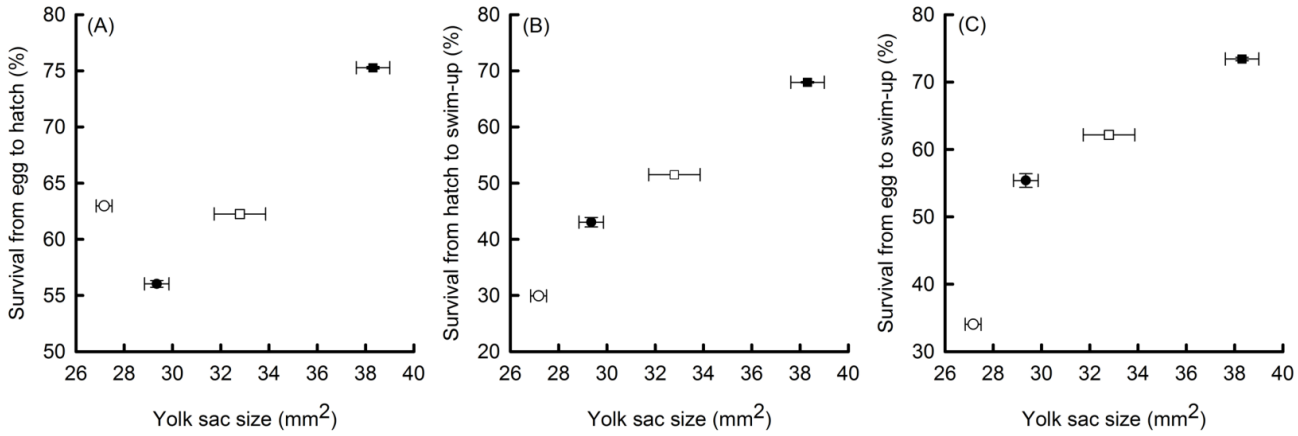


Fig. 4.3. Survival rate of different life history strategies (±SE) during stages of ontogeny. (A) Survival rate from fertililsation to hatch for ferox trout offspring. (B) Survival rate from hatch to swim up stage. (C) Survival rate from egg fertilization to swim-up stage. Ferox trout (FX:FX, closed squares), benthivorous trout offspring (BT:BT, closed circles), female ferox trout and male benthivorous trout (FX:BT, open squares) and male ferox trout and female benthivorous trout (BT:FX, open circles).

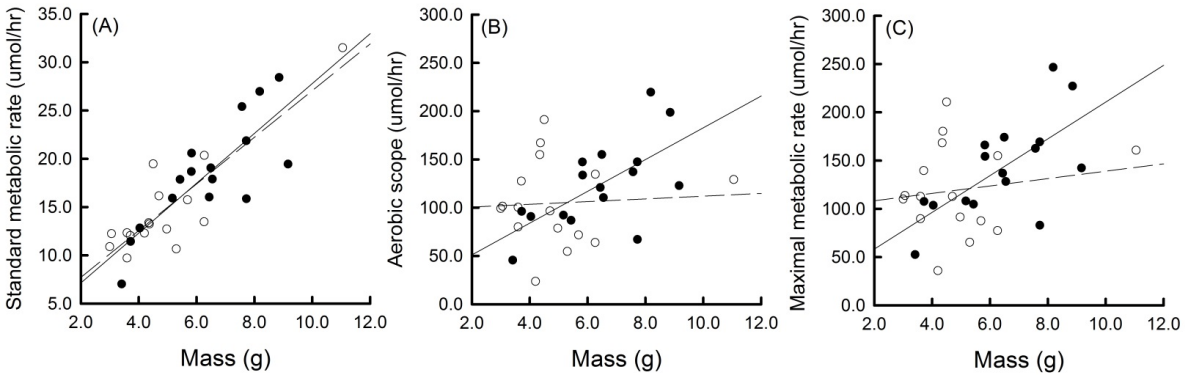


Fig. 4.4. The relationship between metabolic rate (SMR (A), MMR (B) and AS (C)) and mass for ferox trout offspring (closed circles, solid line) and brown trout offspring (open circles, dashed line). See text for statistical analysis but note that there were no significant differences between ferox trout and brown trout offspring in all metabolic measures.

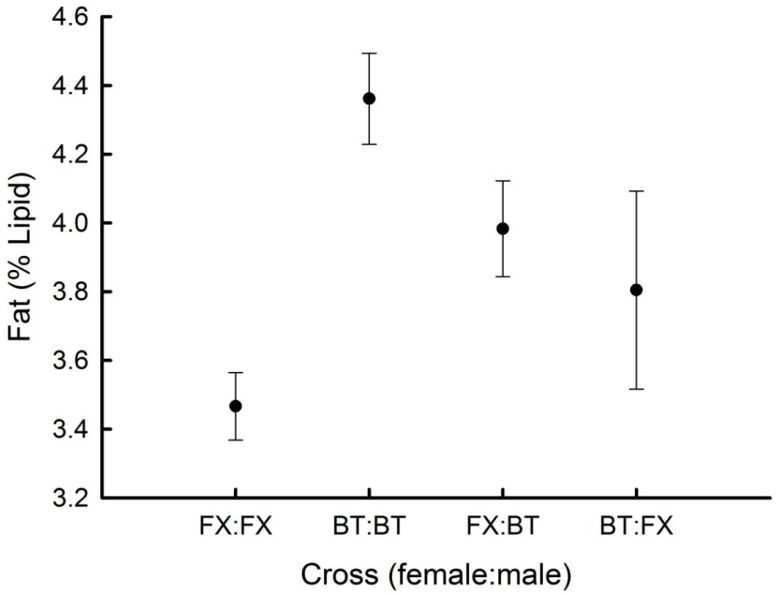


Figure. 4.5. Percentage of lipid reserves (%) in ferox (FX:FX), brown trout (BT:BT) maternal ferox and paternal brown trout hybrids (FX:BT) and paternal ferox and maternal brown trout hybrids (BT:FX) fed on similar amount and diet.

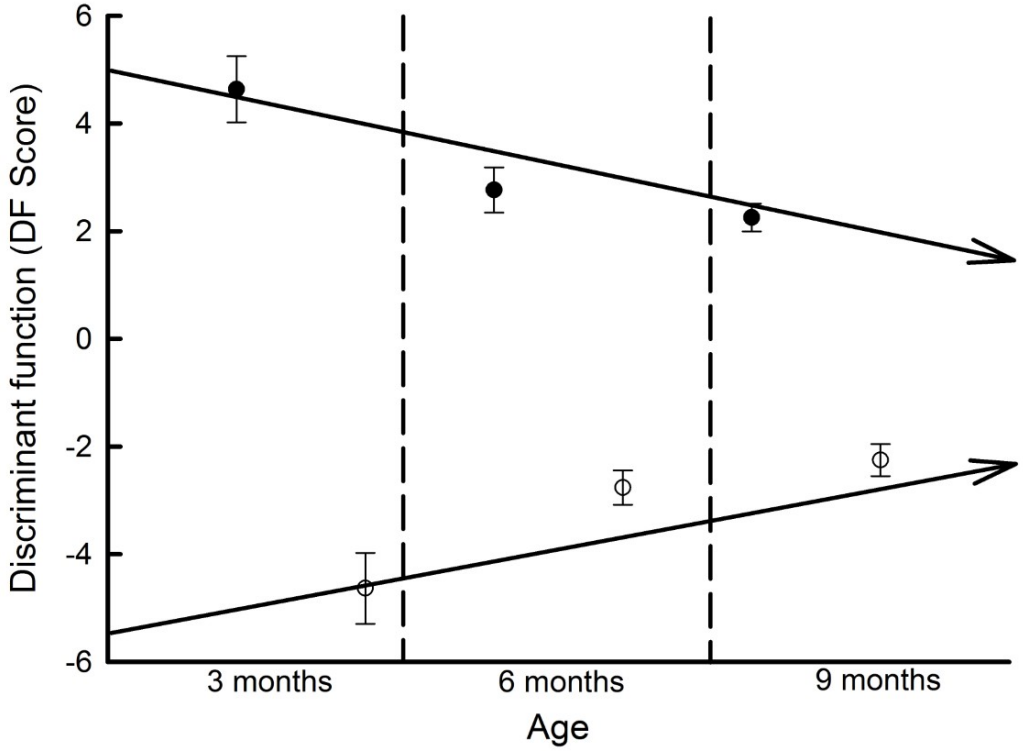


Figure 4.6. Discriminant function (DF) on head shape morphology at 3 months, 6 months and 9 months of ferox trout (solid circles) and benthivorous trout (open circles). Error bars represent standard error of the mean and trend lines are regressions illustrating a convergence on head shape morphology over time.

4.5. DISCUSSION

Stable isotope analysis of eggs from parents collected for our study indicated the presence of two distinct foraging strategies (Fig. 4.1.). Ferox trout eggs had elevated levels of δ^{15}N compared to brown trout eggs. Higher levels of δ^{15}N have been shown to be associated with individuals foraging at higher trophic levels (Grey, 2001) and are similar to previous studies of ferox trout and brown trout in Loch Ness, Scotland (Grey 2002).

Given the larger size of ferox trout it was unsurprising that they produced larger eggs than brown trout, however there was no significant difference in clutch size between the two life history types; thus ferox trout produced a small number of large eggs compared with brown trout which produced a relatively large number of small eggs (Table 4.3.). Despite such variation in egg size, I found no differences in pace of early development stages between hybrids (Table 4.4.). Therefore, this study suggests that there are no obvious maternal advantages between families in embryonic development, however maternal provisioning may still play an important role in subsequent ontogeny. As demonstrated by Leblanc *et al*., (2011) in Arctic charr, egg size alone may influence the life history pathway

of an individual through non-genetic effects. An important maternal provision, related to egg size, is the size of alevin yolk sac (Wallace & Aasjord, 1984). Most embryonic fish rely on yolk reserves as the sole source of nutrition until exogenous feeding can begin, thus the size of yolk reserve may be an important determinant of survival (Braum, 1967; Sargent *et al*., 1986). Large yolk sacs have been shown to decrease risk of starvation (Theilacker, 1981; Gisbert *et al*., 2000) and reduce size related predation risk (Sheperd *et al*., 2000). Ferox trout possessed the largest yolk sacs, with paternal ferox hybrids possessing the smallest yolk sacs (Fig. 4.2.).

Ferox trout offspring had higher survival rates during early development than all other life history types (Table 4.5.). The poor survival rate of hybrids, particularly paternal ferox hybrids (BT:FX), may be an artefact of small yolk reserves. However, the higher mortality of both types of hybrids suggests a level of hybrid inviability between ferox and brown trout, although rigorous testing of hybrid viability is beyond the scope of this paper. It is likely both maternal and genetic effects are contributing to the low survivorship in the hybrid crosses.

Against my prediction I found no differences in SMR, MMR or AS between ferox trout and brown trout offspring (Fig. 4.3.). This is surprising, given the large differences in growth potential found between parental ferox and brown trout (Mangel & Abrahams, 2001). This important physiological mechanism has been shown to affect important ontogenetic stages in juvenile salmonids. Metcalfe *et al*., (1995) demonstrated juvenile salmon with high SMRs were more dominant and thus a higher social status than salmon with low SMRs. As high social status has been linked to smolting (the physiological process in which juveniles modify their internal morphology to adapt to saline water) variation in SMR directly influences life history strategies in juvenile *S. salar* (Metcalfe *et al*., 1989, 1990). Increased dominance and food acquisition, has been previously demonstrated with juvenile ferox trout (Hughes *et al*., 2016). Surprisingly however, I found no difference in SMR, MMR or AS between juveniles from ferox trout and benthivorous trout origin after 9 months of exogenous feeding. Related to metabolism is an individual's lipid content which acts as an energy store. Lipid reserves in salmonids have also been shown to influence important life history stages such as maturity (Rowe & Thorpe, 1990; Rowe *et al*., 1991. In Atlantic salmon, lipid levels in male parr have been shown to be a prerequisite to maturation and smolting (Rowe *et al*., 1991) and important for returning adult Atlantic salmon, where a lipid level threshold must be attained for adults to spawn that season. Remarkably, although there was no measurable difference in metabolic rates, there was a significant difference in lipid deposition between juveniles from alternate life history parentage. Progeny of ferox trout

parents had lower lipid levels than juveniles from benthivorous parents, with hybrids falling between the range (Fig. 4.4). Although all fish were fed on the same type and amount of food, lipid was assimilated at different rates. Based on previous literature, juveniles which accumulate higher reserves of lipid are more likely to reach maturity quicker, or even smolt (Rowe *et al*., 1991; Thorpe *et al*., 1998). Given that ferox trout have been found to delay maturation (Campbell, 1979), this result suggests that progeny of ferox trout are investing less into maturity and more into processes such as growth and territorial defence (Hughes *et. al.* 2016) in early ontogeny than progeny of benthivorous trout. As the lipid reserves of hybrids falls between the ranges, this also suggests accumulation of lipids is, at least in part, derived from parental origin.

Head morphology varied between ferox trout offspring and brown trout offspring across all four measurement periods of early development (Hatch: 3 months; 6 months old and 9 months old; Fig. 4.5.). Although the functional ability of each head shape was not tested here, differences in head morphology during early development (despite the absence of variable environmental factors) suggests a level of inherited head shape differences. Interestingly, differences in head shape morphology between benthivorous and ferox trout reduced over time in a common environment (Fig. 4.5.). This suggests that inherited differences in head shape of juveniles may be lost quickly due to plasticity exhibited by both life history types converging on a more similar shape to suit their environment. Given the large body of literature demonstrating the importance of head morphology for foraging - usually exhibited as trophic polymorphisms in animals expressing alternative life history pathways (McPhail, 1984; Skúlason *et al*., 1989; Adams *et al*., 2003) - significant differences in head morphology here suggests preparation of alternative life history pathways, even during the early stages of ontogeny.

The results of this study suggest differences in important traits which may contribute to an individual's life history pathway exist in offspring of parents from alternate life histories. Important traits such as egg and yolk sac size provided by the maternal line suggest maternal effects may influence juvenile life history - something that has been previously demonstrated (Leblanc *et al*., 2011). However, I cannot discount a genetic basis and/or a combination of both genetic and maternal effects that may be driving the differences observed. Although the importance of maternal effects beyond early development has been questioned (Lindholm *et al*., 2006), this study suggests maternal effects do, in part, influence juvenile traits which may be important for subsequent life history adoption.

With explicit reference to the brown trout/ ferox trout taxonomic debate, this study does not provide a definitive answer. Although brown trout and ferox trout varied

considerably in lipid deposition and head shape morphology, they did not differ in important physiological mechanisms such as metabolism and pace of embryonic development. Interestingly though, it should be noted that ferox x brown trout hybrids often fell between the ranges of measurable traits and suffered high mortalities during early ontogeny, suggesting some level of incompatibility between mature brown trout and ferox trout. Additionally, this study clearly demonstrates that some characteristics considered important to determining an individual's life history pathway, are parentally derived.

CHAPTER 5.

PARENTALLY ACQUIRED DIFFERENCES IN RESOURCE ACQUISITION ABILITY BETWEEN BROWN TROUT FROM ALTERNATIVE LIFE HISTORY PARENTAGE

*Note: A version of this chapter has been published as a manuscript in the journal Ecology of Freshwater Fish Biology, Online version available 28th November 2016.

5.1. ABSTRACT

Dominance hierarchies, where they exist, affect individual food acquisition ability and fitness, both of which have the potential to influence life history pathways. Juvenile salmonids exhibit clear dominance hierarchies in early life. Because one of the drivers for the adoption of alternative life histories in salmonids is the relative rate of resource acquisition, there is potential for juvenile behaviour to influence the subsequent life history strategy of the individual. Lacustrine brown trout, *Salmo trutta*, exhibit a multitude of life histories which includes among others the piscivorous (ferox) life history where individuals grow to large size and have delayed maturity and benthivorous and pelagic life histories where individuals grow to much smaller sizes but mature earlier. Using a number of observable characteristics of dominance this study compared differences in behaviour between size-matched pairs of progeny, reared under common garden conditions but derived from alternative, co-existing life history strategy parents. I found that first generation progeny of ferox trout were more aggressive, acquired more food, had lighter skin pigmentation and held more desirable positions than the progeny of benthivorous brown trout in an experimental stream system. Ferox trout progeny were dominant over benthivorous brown trout progeny in 90% of trials in dyadic contests. Given such clear differences in dominance, this study indicates that parentally acquired dominance related differences, passed through either, or both, of genetic and non-genetic (e.g. maternal effects) means are likely a contributing factor to the continued maintenance of distinct life history strategies of brown trout.

5.2. INTRODUCTION

Individual behaviour can determine mating and reproductive success (Dewsbury, 1982; Cowlishaw & Dunbar, 1991), social status (Gilmour *et al*., 2005), and the quality of resources (Nakano, 1995) and territories acquired (Wells, 1977; Fox & Myers, 1981). Thus an animal's individual behaviour plays a significant role in successful development, longevity and overall fitness. Aggression and territoriality are commonly displayed in

animals where, individuals contest a limited resource in an ecosystem, with successful individuals frequently being the most dominant (Ellis, 1995). Such contests can also influence the social rank of animals and frequently result in hierarchies within a population. Such dominance hierarchies are found across taxa including birds (Ens & Goss-Custard, 1984; Dingemanse & Goede, 2004), fish (Abbot *et al*., 1985; Metcalfe *et al*. 1989), mammals (Seyfarth, 1976; Creel, 2001) and invertebrates (Cole, 1981; Röseler *et al*., 1984) with more dominant individuals generally better at acquiring resources than subordinate individuals. In birds, dominant American redstarts, *Setophaga ruticilla* (L.) regularly exclude subordinates from high quality habitats and are therefore generally of a larger size as a result of the increased food acquisition (Marra & Holmes, 2001). In mammals, dominance rank influences both food access and breeding success. For example, in Red deer, *Cervus elaphus* (L.) higher ranking individuals have greater reproductive success and acquire higher quality resources than subordinates (Appleby, 1980; Clutton-Brock *et al*., 1986). Amongst fish, competition for feeding territories in stream dwelling juvenile salmonids has proven a useful model in the study of social dominance (Metcalfe *et al*., 1989; Huntingford *et al*., 1990; Adams *et al*., 1998; Cutts *et al*., 2001; Höjesjö *et al*., 2005; Burton *et al*., 2011; Preston *et al*., 2014; Van Leeuwen *et al*., 2015).

In juvenile Atlantic salmon, *Salmo salar* (L.), aggression and dominance correlate positively with territory quality and food acquisition, with dominant individuals more likely to achieve higher growth and faster development. This has, in turn been shown to influence the life history of the individual (Metcalfe *et al*., 1998). For example, dominance status has been shown to influence the age at which juvenile Atlantic salmon undergo smolting (the physiological and morphological preparation for salmonids to enter sea water) and thus migrate into marine waters, with dominant individuals migrating at a younger age than subordinates (Metcalfe *et al*., 1989, 1998).

Juvenile brown trout, *Salmo trutta* (L.), display similar dominance hierarchies to those displayed in the closely related, Atlantic salmon (Harwood *et al*., 2002). They also exhibit a multitude of life history strategies with some individuals remaining in natal streams their entire life (river residency), while others migrate to lakes (adfluvial potamodromy) or into marine waters (anadromy). Life history strategy is partly dependent on the life history of the parent (through genetic and non-genetic maternal effects; Van Leeuwen *et al*., 2015) and the relative rates of resource acquisition (see review by Dodson *et al*., 2013), which is likely dependent on the behaviour of the individual. One understudied life history of *S. trutta* is the ferox life history pattern. Ferox trout manifest as lacustrine

dwelling, piscivorous trout (Grey, 2001; Grey *et al*., 2002) which grow to large size (Mangel & Abrahams, 2001), exhibit delayed maturation (Campbell, 1971) and increased longevity (Mangel & Abrahams, 2001). Considered one of the many life history types adopted by the highly variable *S. trutta* species complex, ferox trout are associated with large, deep oligotrophic lakes and the presence of Arctic charr *Salvelinus alpinus* L. (Campbell, 1979; Greer, 1995; Hughes *et al*., 2016). Ferox trout have also been described as reproductively isolated and genetically distinct from brown trout expressing other life history traits in sympatry in some lakes in Scotland and Ireland (Ferguson & Mason, 1981; Ferguson & Taggart, 1991; Prodöhl *et al*., 1992; McVeigh *et al*., 1995; Duguid *et al*., 2006). As a result, ferox trout have been variously classified as either one of the many adopted life history types or as a distinct species *Salmo ferox* Jardine, 1835; a nomenclature recognised by the International Union for the Conservation of Nature (IUCN) (Freyhof & Kottelat, 2008).

Given that the adoption of piscivory in fishes is often limited by an individual's gape size (Persson *et al*., 1996; Mittelback & Persson, 1998), increasing growth through food acquisition to reach a critical size threshold at which they can access fish prey would benefit piscivorous species by enabling them to exploit larger prey items (and thus fish prey) at an earlier developmental stage. Therefore, given the advantages of dominance rank on food acquisition in juvenile salmonids, I hypothesise that if the adoption of a life history pathway is parentally derived, either genetically or through maternal effects, then juvenile progeny of ferox trout will be more dominant than progeny of brown trout, adopting the more common foraging strategy of lacustrine trout exploiting macrobenthic invertebrates (hereafter benthivores).

To test for differences in dominance related traits between life histories, I reared progeny of sympatric ferox and benthivorous brown trout in the laboratory under common garden conditions and compared commonly used indicators of dominance behaviour (aggression, skin colour, spatial position and food acquisition) in size-matched, dyadic contests in an experimental stream.

5.3. Materials And Methods

5.3.1. Broodstock Collection

Broodstock 3 ferox females and 3 ferox males, 3 benthivorous brown trout females and 3 benthivorous brown trout males) were captured using fyke nets and electrofishing between 1 October and 12 November 2013 from two tributaries in the Loch Maree catchment, Scotland. Reciprocal hybrid crosses between a male ferox and female benthivorous brown trout and a female ferox trout with a male benthivorous brown trout

were made. However due to high mortality of hybrids during early development, they could not be used for this experiment. For future studies the high mortality of hybrids crosses should be noted, and may infer a level of hybrid inviability. Due to the rarity of ferox trout (Duguid *et al*., 2006), the likelihood of collecting ripe females during spawning time is low. At capture, fish were classified as ferox trout or benthivorous brown trout based on size: ferox trout (40-80cm); benthivorous brown trout (20-35cm) (Campbell, 1979) and prior knowledge from local angling groups about spawning locations. Classification of the two life history types was confirmed by subsequent stable isotope analysis of egg samples. Mature fish were transported to holding tanks on the Coulin Estate, Kinlochewe, Scotland where they were held in two large, 2000 L tanks supplied with river water and assessed daily for ripeness. On 14 November 2013, all fish were anaesthetized, blotted dry, and their eggs or sperm extruded by abdominal massage. Eggs were fertilized by randomly selected males of the same life history type to create two full sibling families of each life history.

5.3.2. Egg Rearing And Animal Husbandry

Fertilised eggs were transported to the Scottish Centre for Ecology and the Natural Environment (SCENE), Loch Lomond, Scotland. Each family was reared separately in mesh baskets held in clear plastic tanks (50cm X 30cm X 15cm) in a temperature controlled environment chamber (temp $7.4 \pm 0.1^{o}C$) using water on a partial recirculation system. Water was pumped directly from Loch Lomond before being channeled through a free-standing filter unit and a single in-line UV sterilizer. Eggs were held in complete darkness until hatching. Eggs were examined daily, with any dead eggs carefully removed. Egg size (two dimensional surface area (mm^2)) was measured using image software (ImageJ) from photographs of eggs taken 48 hours after fertilization. The timing of emergence of successive developmental stages: eye pigmentation, hatch and yolk absorption, was recorded as the number of degree days (DD) (calculated as the sum of the daily mean water temperature each day) since fertilization. Ferox trout eggs hatched from 5 January to 8 January 2014, with eggs from benthivorous brown trout hatching from 7 January to 11 January 2014. Following hatching, alevins were raised under an ambient photoperiod. After complete yolk absorption, fry were fed a standard commercial salmon pellet (Biomar, Aarhus, Denmark) at approximately 3% body wt. day^{-1}. On 5 April 2014, fish were moved to larger 175 L radial flow circular tanks. At this stage, families were mixed to create two groups of equal density (160 fish in each of the 2 tanks) for each life history.

5.3.3. Stable Isotope Analysis

Stable isotope analysis was conducted to confirm the different foraging strategies of the broodstock used. Previous stable isotope analysis on ferox trout in Loch Ness, indicated ferox trout would have significantly elevated δ^{15}N signature compared to benthivorous brown trout feeding on zooplankton or macroinvertebrates (Grey, 2002).

Eggs (n=4) from each family were randomly selected from each batch during stripping (total n=16) and dried for 96 hours at 48°C in a drying oven. The dried tissue was ground to a fine powder using a pestle and mortar. Approximately 50% of each sample was then lipid extracted as follows: 15 mg of ground tissue was soaked in a 2:1 (by volume) chloroform:methanol solvent mixture. After 20 minutes, the sample was centrifuged (3000 rpm for 5 minutes), the supernatant discarded and the process was repeated until the solvent ran clear. The lipid extracted samples were then dried for a further 96 hours at 48°C in a drying oven. Non-lipid extracted and lipid extracted samples (n=32) were measured (7-9 mg) into tin capsules (standard weight 5 x 3.5mm). Carbon (δ^{13}C) and nitrogen (δ^{15}N) stable isotope ratios were determined by continuous flow isotope ratio mass spectrometry (CF-IRMS), using a Costech ECS 4010 elemental analyser coupled to a ThermoFisher Scientific Delta XP-Plus IRMS at the NERC Life Sciences Mass Spectrometry Facility.

Experimental fish were introduced into one of fifteen compartments (60 cm x 60 cm x 60 cm) of an artificial flume channel at the Scottish Centre for Ecology and the Natural Environment, Rowardennan, Scotland. Each compartment was partitioned by plastic mesh mounted to a wooden frame. Homogeneous substrate (gravel/pebble) was distributed throughout each compartment with a single (10 cm x 5 cm) rock located in the centre of the arena. The rock was used as an indicator of optimal habitat for the experimental fish as juvenile salmonids have been shown to hold central positions in streams behind such structures (Metcalfe *et al*., 2003). A 30 cm tube terminated immediately upstream of this central position into which food pellets were introduced (so increasing the likelihood of competition between the 2 fish). The observation area, located in the centre of the flume, allowed observers to view the fish behind glass without interfering with the fishes natural behaviour (Fig. 5.1).

5.3.4. Behavioural Trials

Dyad behavioural trials were conducted using one individual of ferox origin and one of benthivorous brown trout origin. As dominance in salmonids has been shown to be significantly affected by body size (Huntingford *et al*., 1990), fish pairs were size matched

by length to the nearest mm to reduce any size effects and potentially any residual maternal nutritional effects. One fish from each pair was randomly marked with alcian blue on the dorsal fin to allow distinction between individuals in each compartment to be made. Thirty pairwise trials were conducted over a 3-week period. Once introduced to the arena, the fish pair were left for 48 hours to acclimate. Food pellets were introduced five times daily throughout this acclimation period via feeding tubes to further accentuate the optimal spatial position in each compartment. Following acclimation, fish were observed four times daily for 6 minutes at 9:00, 11:00, 13:00, 15:00 h over two days. Four behavioural characteristics of dominance in salmonid fish were measured: overt aggression rate, fish colouration, spatial position and food acquisition (Metcalfe *et al*., 1989; Adams *et al*., 1998; Nicieza & Metcalfe, 1999; Adams *et al*., 2000; Kittilsen *et al*., 2009).

Aggressive interactions, fish colour and spatial position were scored during an initial 3-minute period during each observation. After this 3-minute period, a single food pellet was introduced to the chamber and the acquisition of this food item by either fish recorded. A second 3-minute observation followed the introduction of the food pellet during which aggression, fish colour and spatial position were recorded. Fish displaying aggressive behaviour during observations were scored (+1) for each aggressive display. Five common characteristic overt aggressive behaviours were recorded during observations: fin nips, chases, mouth gapes, fin displays and fish displacements (Metcalfe *et al*., 1989; Adams *et al*., 1998; Adams *et al*., 2000; Kittilsen *et al*., 2009).

As body colouration is a well-known indicator of stress in salmonids (Kittilsen *et al*., 2009), the flank colour of each fish was recorded at the beginning and end of each observation. As subordinates tend to be darker in colouration, fish with dark bodies were scored negatively (-1), and those with lighter colouration scored positively (+1), intermediate colouration received a score of (0).

The behavioural arena was marked into three equal sized units in the x, y and z dimensions, using marbles on the substrate and markings on the observer glass, to indicate partition boundaries. Thus a total of 27 cuboids within each chamber were defined and each allocated a score according to their proximity to the central optimal location. Cuboids immediately below the feeding tube above the substrate were classed as ‘optimal’ locations and were given a positive score (+1). Cuboids on the periphery of ‘optimal’ locations were neutrally scored (0) with cuboids located in the corners of the arena being scored negatively (-1). In addition to this, fish observed lying on the substrate or mesh partition received an

additional negative score of (-1). If fish were highly active during observations every visited cuboid score was recorded and an average calculated to provide a single score for each fish.

Food acquisition following pellet introduction was scored as follows. If a fish made no attempt at acquiring the food pellet, it scored 0, if a fish attempted to acquire the food pellet but was unsuccessful, it scored +1, a fish that was successful in acquiring the food pellet scored +2.

5.3.5. Statistical Analysis

Differences in egg size and the timing of developmental milestones between offspring of each life history parentage, were tested using ANOVA

To test for differences in mean $\delta^{13}C$ and $\delta^{15}N$ ratios between ferox trout and benthivorous trout eggs, a Welch's t-test was conducted on both lipid extracted and non-lipid extracted values.

To test if the outcome of the number of winning contests between ferox trout and benthivorous brown trout were equally distributed, a chi-square goodness-of-fit test was conducted. Normality and homogeneity of data were tested using the Shapiro-Wilk test and the Anderson-Darling test, respectively. Due to a failure of normality, non-parametric tests were used in subsequent analysis. Correlations between the four variables (aggression, skin colour, spatial position and food acquisition) were described using a non-parametric Spearman's Rank Correlation test, before being summarised with a principal component analysis (PCA). An overall dominance score was obtained by extracting PC scores for each fish from PC1 which weighted positively for aggression, colour, spatial position and food acquisition (Table 5.3.). To test univariate aggression rates, food acquisition rates, colour index and spatial scores between ferox trout and brown trout, a non-parametric Mann-Whitney *U*-test (two sample Wilcoxon rank sum) was used. Dominance scores were compared using a Welch two sample t-test. All statistical analysis was performed using R version 3. 1. 2 statistical software (R Core Team, 2014).

5.4. Results

Brown trout eggs (n = 8) were significantly more depleted in $\delta^{15}N$ (t = -35.4, df = 13.1, P <0.01) than ferox trout eggs (n = 8). There was no significant difference in $\delta^{13}C$ (t = 1.4, df = 12.3 P = 0.2) between brown trout and ferox trout eggs (Fig. 5.2.) Similarly lipid extracted samples from brown trout eggs (n = 8) were significantly more depleted in $\delta^{15}N$ (t = -35.2, df = 13.8, P <0.01) than ferox trout eggs (n = 8). and there was no significant difference in $\delta^{13}C$ (t = 0.2, df = 12.8, P = 0.84).

There was a significant difference in egg surface area (mm^2) between life history types ($F_{1, 197} = 82.06$, $P < 0.001$) with ferox trout progeny having a greater egg surface area than brown trout progeny ($P < 0.001$) (Table 5.1.).

There was no significant difference in development time taken to reach successive stages (time to eyed stage ($F_{2,3} = 1.1$, $P = 0.51$); time to hatch ($F_{2,3} = 1.1$, $P = 0.51$); time to swim-up ($F_{2,3} = 6.5$, $P = 0.13$)) between offspring types (Table 5.2.).

There was a strong, positive correlation between all four observed indicators of dominance (Table 5.3.). PC1 weighted all four variables highly and positively and summarised 58% of the variation. Thus high PC1 scores characterised individuals with high levels of aggression, lighter skin colouration, held better spatial positions in the arena and greater rates of food acquisition. Ferox trout progeny had the higher dominance score of the pair (based on PC1) in 27 of 30 trials. This is greater than would be expected by chance ($\chi^2 = 41.85$, $df = 1$, $n = 60$, $P < 0.01$). The mean aggression rate of ferox trout progeny was 14.8 ± 2.51 (mean ± SE) compared with benthivorous brown trout progeny 5.0 ± 1.32 (mean ± SE; Mann-Whitney *U*-test: W = 222.0, $P < 0.01$). The mean food acquisition rate of ferox trout progeny was 5.6 ± 0.82 (mean ± SE) higher than benthivorous brown trout progeny 3.2 ± 0.76 (mean ± SE; Mann-Whitney *U*-test: W = 294.0, $P < 0.05$). The mean spatial position score of ferox trout progeny was 5.3 ± 1.17 (mean ± SE), compared with -15.0 ± 2.76 (mean ± SE) for benthivorous brown trout progeny (Mann-Whitney *U*-test: W = 94.5, $P < 0.01$). The mean body colouration score of ferox trout progeny was 11.4 ± 1.03 (mean ± SE), compared with -3.43 ± 1.77 (mean ± SE) for benthivorous brown trout progeny (Mann-Whitney *U*-test: W = 83.5, $P < 0.01$). The mean dominance score of ferox trout progeny was 1.04 ± 0.10 (mean ± SE) compared with -1.04 ± 0.17 (mean ± SE) for benthivorous brown trout progeny (Welch two sample t-test $df = 45$, $P < 0.01$) (Fig. 5.3.).

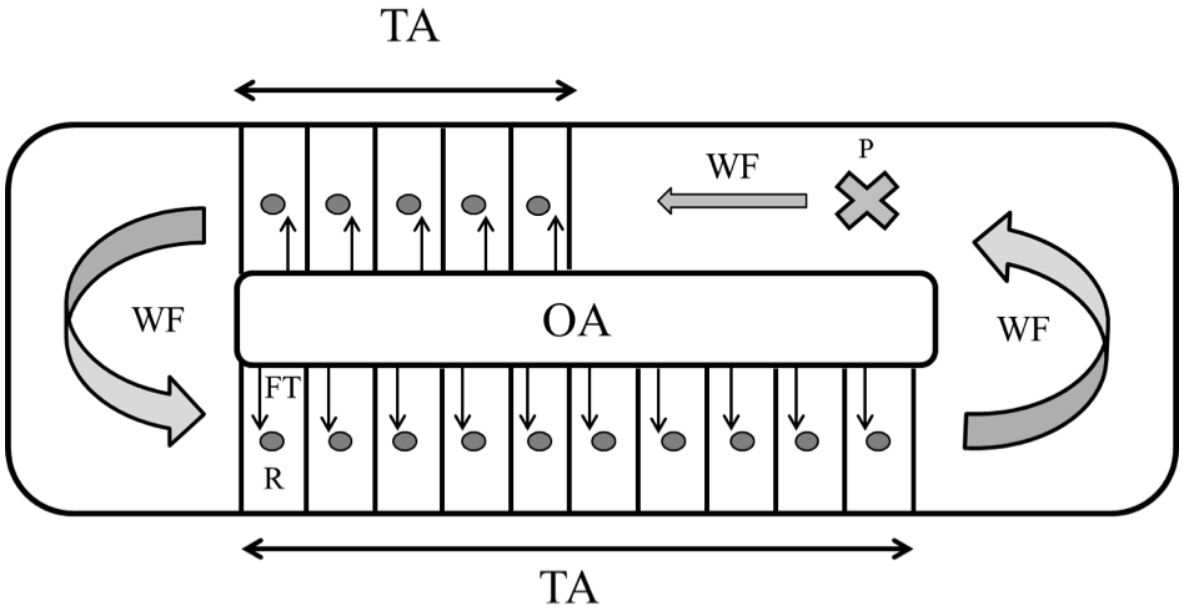


Figure 5.1. Schematic diagram of the artificial stream used for fish observations. Trial arenas (TA), feeding tubes (FT), rocks (R) used for indicator of prime habitat, propeller (P), water flow (WF) and central observation area (OA).

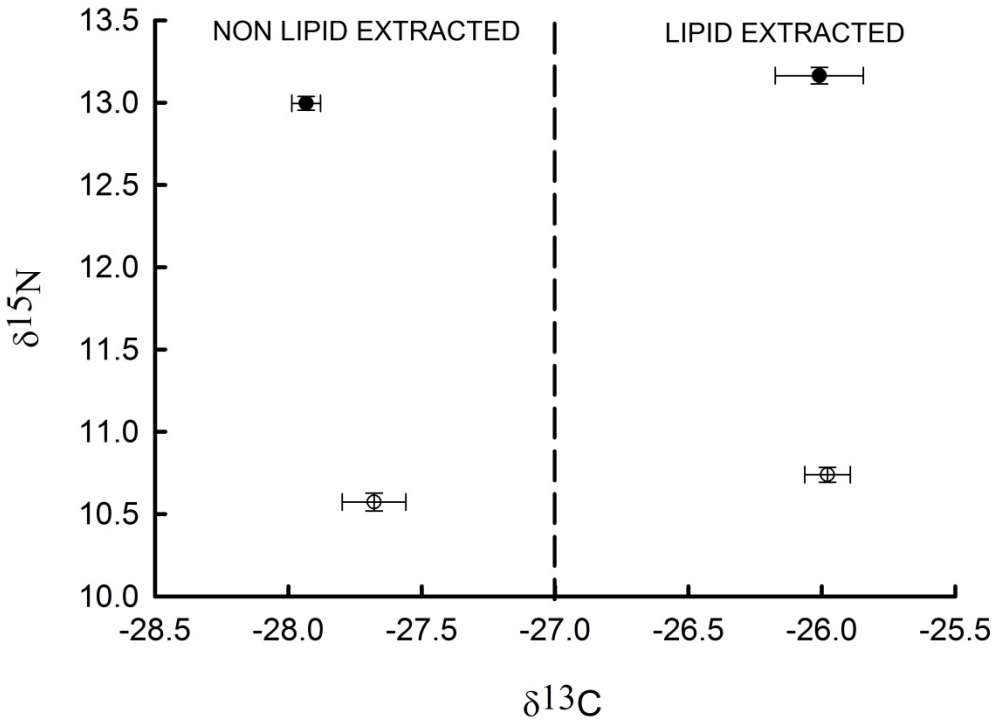


Figure 5.2. Mean (± SE) table isotope ratio of carbon and nitrogen in benthivorous trout (○) and ferox trout eggs (●) from the Loch Maree catchment.

Table 5.1. Egg number and egg surface area (mm^2 ± S.E) of each family of offspring.

Family	Life History	Egg Number	Egg Area (mm^2) ± S.E.
1	FX	122	30.67 ± 0.17
2	FX	267	32.45 ± 0.18
5	BT	328	30.44 ± 0.19
6	BT	462	26.83 ± 0.21

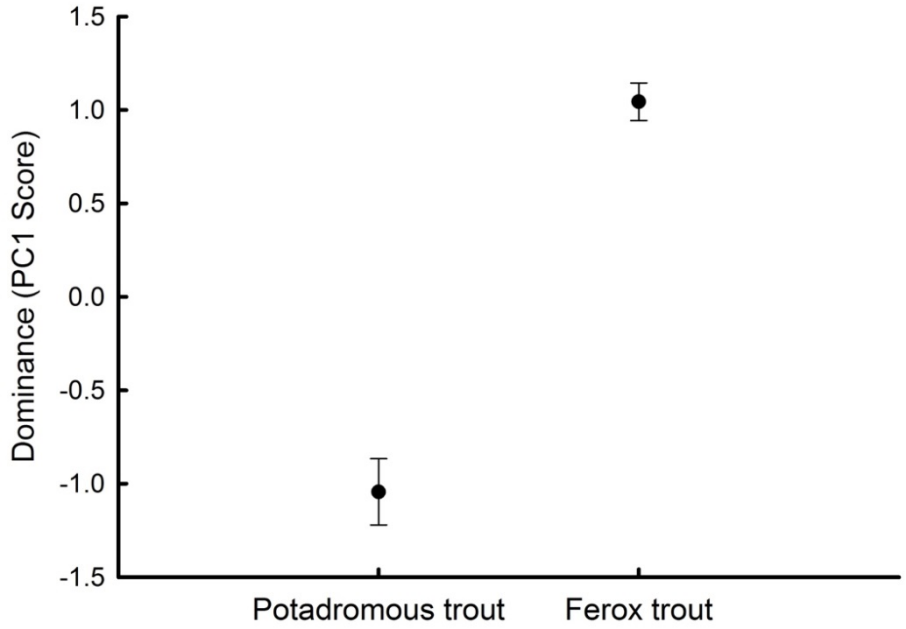
Table 5.2. The developmental time (measured in degree-days (^oC.day) of three key developmental stages: eye pigmentation present in eggs, day of hatch and 'swim-up' stage.

Family	Life History	Eyed-egg stage	Hatch	Swim-Up
1	FX	239.1	492.9	852.2
2	FX	239.1	468.9	843.2
5	BT	227.5	484.8	869.4
6	BT	239.1	461	861

Table 5.3. Pairwise Spearman's correlation coefficients and PC1 coefficients from PCA analysis, for all four behavioural traits observed. All four traits were significantly correlated with one another (P <0.01), with the first principal component summarising 58% of the variation.

	Behavioural traits				
	Aggression	Colour	Position	Food	PC1
Aggression		$P < 0.01$	$P < 0.01$	$P < 0.01$	0.4
Colour	0.51		$P < 0.01$	$P < 0.01$	0.57
Position	0.37	0.71		$P < 0.01$	0.56
Food	0.46	0.38	0.48		0.44

Figure. 5.3. The mean (± standard error) extracted PC1 scores (overall dominance score) PCA analysis of 4 variables (aggression, colour, spatial position and food acquisition) of benthivorous brown trout and ferox trout offspring.



5.5. DISCUSSION

This study demonstrates a very clear difference in dominance behaviour in juvenile *S. trutta* derived from parents expressing two alternative life history strategies; a piscivorous, ferox life history strategy and a benthivorous life history strategy. Although all experimental

fish were reared at the same densities, fed the same type and quantity of food and were size matched prior to behavioural observations, the progeny of ferox trout parents were more aggressive, acquired more food and held more advantageous spatial positions in the stream than benthivorous brown trout offspring. Thus overall, progeny of ferox trout exhibited considerably higher levels of dominance than the progeny of benthivorous trout in the experiment.

Given that all individuals in this experiment were reared from eggs under common conditions, the high levels of dominance exhibited by the progeny of ferox trout, compared with trout of benthivorous parental origin, indicates that the expression of behavioural dominance was parentally acquired.

As the adoption of a piscivorous diet in many fish species, including *S. trutta*, is dependent upon gape size, it is generally thought that individuals must achieve a minimum size threshold before they may access fish prey (Campbell, 1979; Persson *et al*., 1996; Mittelback & Persson, 1998).

The wealth of literature on salmonids shows that the dominance rank expressed has consequences for the life history pathway ultimately adopted in later life (Metcalfe *et al*., 1989, 1998). High dominance status individuals acquire higher quality territories and take a greater share of resources, and in particular exhibit higher levels of food acquisition, than those of lower dominance status. Thus in conditions of limited food availability salmonid fish exhibiting high behavioural dominance, grow faster than those of lower dominance (Wells, 1977; Metcalfe *et al*., 1989; Nakano, 1995; Adams *et al*., 1998; Cutts *et al*., 2001; Höjesjö *et al*., 2005; Van Leeuwen *et al*., 2015).

One consequence for *S. trutta* individuals for which the piscivorous, ferox life history is a possible strategy is that if foraging resources are limited, then high dominance status is likely to result in higher growth and the attainment of the size threshold for piscivory at an earlier age than would be possible for individuals expressing low dominance status. Thus one logical conclusion is that *S. trutta* adopting a ferox life history strategy are more likely to be drawn from individuals with a high dominance status, allowing them to acquire a higher share of the foraging resources and thus reach the size threshold for piscivory more quickly.

That *S. trutta* adopting a ferox life history strategy have a higher dominance status than those adopting a benthivorous life history strategy was not tested in this study. The first generation offspring of parents adopting a ferox life history strategy were however very clearly and consistently expressing a higher dominance status than the first generation offspring of parents adopting a benthivorous life history strategy.

The mechanism through which behavioural dominance status was acquired from their parents was not clearly identified in this study. There are however, at least two alternatives that are not mutually exclusive. Dominance status may be genetically inherited, with the behavioural traits that confer dominance simply passed to offspring from their parents. This explanation is certainly plausible as inheritance of foraging behaviour is well known from other species (Ferguson & Noakes, 1982, 1983; Kamler, 2005). Supporting this explanation is evidence for at least some populations, that *S. trutta* exhibiting a ferox life history strategy are genetically distinct from those adopting a benthivorous life history strategy, when the two are in sympatry (Ferguson & Mason, 1981; Ferguson & Taggart, 1991; Prodöhl *et al*., 1992; McVeigh *et al*., 1995; Duguid *et al*., 2006). It may also be noted that as well as being genetically distinct and thus reproductively isolated in sympatry, all ferox so far examined in sufficient detail in Ireland and Scotland are derived from the same lineage (McKeown *et al*., 2010, and references therein). Thus selection may have occurred in an ancestral population and not convergently in current ones. If this is the mechanism for cross-generational transmission of behavioural traits resulting in dominance, this suggests that these traits have been positively selected for in populations that express high proportions of the ferox life history strategy and less so (or not at all) in populations expressing a high frequency of the benthivory life history strategy. There are currently no population genetics data for *S. trutta* expressing the two life history strategies from the Loch Maree catchment to provide support (or not) for this possible mechanism.

Alternatively, dominance traits may be transmitted across generations through maternal effects. In the experiment reported here, I tried to control for maternal effects on dominance operating through body size by matching individuals, as size is known to be strongly correlated with dominance in salmonids (Huntingford *et al*., 1990). Despite this, it is highly plausible that, some maternal effects that I did not control for may be responsible for the transmission across generations reported here. Leblanc *et al*., (2011) for example showed that maternally derived egg size in Arctic charr, *Salvelinus alpinus* influences juvenile behaviour, morphology and foraging activity. There was a difference in egg size between life history origins, in the experiment reported here, and it is thus conceivable that although juveniles were size matched in the dyad experiment, that some egg size or nutritional legacy was conferred on one life history group that did not accrue in the other.

This study demonstrates at least one trait driving the maintenance of the ferox life history type is inherited from one generation to the next. Given that ferox trout are rare (Duguid *et al*., 2006), live in low densities in the wild (Thorne *et al*., 2016) and are highly sought after by recreational anglers, these findings have implications for the management of

this rare life history type. As most international conservation policy is focussed at the species level, less protection is afforded to populations or specific life history types, as a component part of a larger species complex. Therefore, conservation strategies risk overlooking important rare phenotypes, such as ferox trout. The inability to agree on a taxonomic classification for ferox trout among anglers, fisheries managers and academics as either an adopted life history within the broader *S. trutta* species complex or as a distinct species *S. ferox*, has important conservation implications (Ferguson, 2004; Freyhof & Kottelat, 2008). I therefore propose that conservation strategies must include consideration of the complex natural processes which give rise to these rare life history types to effectively protect the full range of biodiversity for the future.

CHAPTER 6.

WITHIN LAKE MOVEMENT OF SYMPATRIC BROWN TROUT *SALMO TRUTTA* ECOTYPES

6.1. ABSTRACT

Differences in spatial and temporal behaviour between sympatric ecotypes is an important mechanism contributing to incipient speciation in animals. Numerous ecotypes, in freshwater fish inhabiting post-glacial lake systems, have been described. Recent acoustic telemetry studies have revealed some ecotypes exhibit differences in home range, core area and activity within a single ecosystem. I examined important movement patterns between two *Salmo trutta* ecotypes, pelagic and benthic, in a remote oligotrophic lake in County Donegal, Ireland. No differences in home range (MCP_{100}/KUD_{95}) or core area (KUD_{50}) were found between benthic and pelagic *S. trutta* throughout the study. Time of day was found to have a significant effect on the activity rate with both ecotypes switching from nocturnal to diurnal activity cycles throughout the study period. Benthic trout peaked in activity around midday in September. Both ecotypes utilised the single outflowing river, the River Finn during the study. Patterns of habitat use overlapped extensively between both ecotypes throughout the study, however pelagic fish were commonly found in the east basin of the lake, over deep waters. Both forms responded similarly to temporal change which suggests environmental parameters heavily modulate movement patterns in *S. trutta* in this lake, regardless of foraging behaviour. In addition, the use of the out flowing river by mature lacustrine *S. trutta*, outside of the spawning season, is an important observation for fishery managers.

6.2. INTRODUCTION

The movement of fish within marine and freshwater systems may be influenced by a number of factors such as water discharge (Feunteun *et al.,* 2000), water temperature (Jonsson & Jonsson, 2002; Biro *et al*., 2009), an individual's foraging behaviour (Barry *et al*., 2015) and even lunar phase (Henderson *et al*., 2014; Barry *et al*., 2015). Measuring the movement of a fish through space and time may reveal important environmental factors influencing an individual's behaviour, useful to both fishery managers and ecological scientists investigating the underlying mechanisms driving incipient speciation.

Acoustic telemetry studies have been used to examine important large and small scale fish movements within and across ecosystems such as migratory patterns (Kessel *et al*.,

2014), predation bottlenecks (Welch *et al*., 2009; Newton *et al*., 2016) and spawning habitats (Afonso *et al*., 2009). In smaller, closed systems, like that of lacustrine systems, telemetry studies aid identification of habitat occupancy (residency) (Anras *et al*., 1999), home ranges (Lucas & Bara, 2000) and habitat utilisation often through kernel utilisation distribution (KUD) (Lowe *et al*., 2003) typically measured as KUD_{50} (50% of fish position – core range) and KUD_{95} (95% of fish position – home range).

Numerous ecotypes in fish inhabiting post-glacial freshwater lakes have been described within several species (Skúlason & Smith, 1995; Smith & Skúlason, 1996). These often occur in sympatry with clear morphological and genetic structuring (Robinson & Wilson, 1996; Dynes *et al*., 1999). Such ecotypes have been recorded in Three-spined stickleback *Gasterosteus aculeatus* (Schluter & McPhail, 1992) Arctic charr *Salvelinus alpinus* (Jonsson & Jonsson, 2001) and Lake whitefish *Coregonus clupeaformis* (Kahilainen *et al*., 2006). Partitioning of resources through use of discrete habitats is thought to be an important mechanism contributing to the evolutionary divergence of sympatric ecotypes. Skúlason *et al*., (1999) suggest that secondary to phenotypic change (adaptive morphology), differences in spatial and temporal use of sympatric habitat is one of the key drivers of incipient speciation. Thus, by investigating basic movement patterns in sympatric ecotypes such as home range, core area, residency and diel movement, assumptions about potentially diverging populations can be explored and fundamental ecological and evolutionary hypotheses tested.

Despite such evolutionary importance, within habitat movement of sympatric ecotypes is relatively understudied (Barry *et al*., 2015). However, a recent study by Hawley *et al*., (2016) examined the fine-scale movement of littoral and pelagic *S. alpinus* in a remote lake. Pelagic *S. alpinus* were found to be significantly more active, fed further from the substrate and at greater depths than littoral *S. alpinus*. Habitat use differed between the ecotypes across seasons, and both types exhibited similar monthly fluctuations in home range (KUD_{95}) and core habitat area (KUD_{50}) size.

Brown trout *Salmo trutta* exhibit multiple life history strategies, which include ecotypes diverging across the benthic and pelagic axis (Ferguson & Mason, 1981). In Lough Melvin, Ireland, benthic and pelagic ecotypes have been identified by measurable differences in morphology (Cawdery & Ferguson, 1988). Benthic *S. trutta*, known vernacularly as Gillaroo, tend to have short jaws, a deep caudal peduncle, many vomerine teeth, a long caudal fin, deep body, a long dorsal and short anal fins. Many vomerine teeth found in Gillaroo are believed to aid handling of mollusc prey items during benthic feeding (Cawdery & Ferguson, 1988). Conversely, pelagic or sonaghen trout tend to have short

shallow heads, long anal fins, few vomerine teeth, long pectoral fins and long gill-rakers. Long-gill rakers aid filtration of zooplankton and are found regularly in other pelagic ecotypes (Lindsay, 1981; Kahilainen *et al*., 2011). Interestingly songahen, gillaroo and a third piscivorous ecotype, known colloquially as ferox trout, have been shown to be reproductively isolated in Lough Melvin (Ferguson & Mason, 1981; Ferguson & Taggart, 1991) and as such are recognised by the IUCN as separate species: Sonaghen *Salmo nigripinnis*; Gillaroo *Salmo stomachicus* and ferox *Salmo ferox* (Freyhof & Kottelat, 2008). Another oligotrophic lake in Ireland, Lough Finn, was used in this study to measure the movement pattern of *S. trutta* expressing benthic and pelagic phenotypes similar to those described by Cawdery & Ferguson (1988). The main objectives of this study were to quantify spatial movement of distinct *S. trutta* ecotypes within a lacustrine habitat and measure differences in home range, residency, activity (movement) and habitat use using acoustic transmitters in a fixed array.

6.3. Materials And Methods

6.3.1. Study Area And Reciever Array

Lough Finn is an oligotrophic lake situated in County Donegal, Ireland (54.864°N 8.131°W). The lake measures approximately 5km in length and lies in a general east to west orientation. It has a maximum width of 0.5km with a total surface area of approximately 2.5km^2. Lough Finn has a maximum depth of 21m and there is a single outflowing river located on the east bank of the lake, the River Finn. The fish community is typical of the area, with Atlantic salmon *Salmo salar*, Arctic charr *S. alpinus*, European Eel *Anguilla anguilla* and Brown trout *S. trutta.*

To assess available habitat for fish in Lough Finn, a visual habitat survey was conducted using an underwater camera (Spyball Model SB-MO- Monochrome, Submertec) across a series of intersecting transects. From this survey, coarse habitat descriptions were assigned to regions of Lough Finn.

To ensure continuous detection of tagged fish throughout the study and adequate coverage of acoustic receivers, preliminary range tests were conducted. Results from these range tests provided the basis for deployment of 20 omnidirectional acoustic receivers (69 KHz, VEMCO VR2W) throughout the lake (Fig. 6.1.). Each receiver was secured to a concrete anchor (3m from the lake floor) and fixed in place with a rope riser. Once in place the fixed acoustic array enabled coverage of the entire lake system.

6.3.2. Fish Sampling And Tagging

S. trutta were collected by fyke nets between 1st of July and 2nd July 2013. *S. trutta* were initially classified as either benthic or pelagic by visual observation. Benthic *S. trutta*, tend to have short jaws, a deep caudal peduncle, many vomerine teeth, a long caudal fin, deep body, long dorsal and short anal fin. Conversely, pelagic trout tend to have short shallow heads, long anal fins, few vomerine teeth, long pectoral fins and long gill-rakers (Cawdery & Ferguson, 1988), which was later confirmed by morphometric analysis. Overall, seven benthic and thirteen pelagic individuals were tagged (n= 20 total) with a single uniquely coded 69KHz acoustic transmitters (Model LP-7.3, 7.3mm diameter, 18mm length, 1.9g weight in air, 139dB re 1 μPa power, Thelma Biotel AS, Trondheim, Norway 2013). Transmitters were programmed to each have an average acoustic transmission repeat cycle of 120s. Fish were anaesthetised for the surgical tagging procedure by immersion in a water clove oil solution (0.5mg per litre). After loss of equilibrium each fish was placed in a surgical pillow and an 8mm incision was made anterior to the pelvic girdle. A single acoustic transmitter was inserted into the body cavity before the incision was closed using two independent sterile sutures (6-0 ETHILON, Ethicon Ltd, Livingston, UK). Fish were kept oxygenated throughout the procedure by pumping lake water through the mouth and over the gills during surgery. After insertion of each tag, fish were placed in 5 litres of lake water, which was replenished regularly for each individual. Fish were released in the original capture site after a two-hour recovery period. The lifespan of each tag was approximately 90 days, thus within lake movements were recorded from the 1st or 2nd of July 2013 (dependent on day of capture) to 30 September 2013.

6.3.3. Head Shape Analysis

Morphometric data collected on tagged brown trout was pooled with morphometric data from a previous study. In the previous study 117 brown trout were sampled using Nordic gill nets set at various depth and habitats. Stomachs were removed by dissection and prey identified to family level and where possible species level. Brown trout were assigned to one of two putative groups called benthic (if their stomach contents consisted of 50% or more benthic prey items) or pelagic (if their stomach contents consisted of 50% or more pelagic prey times. A principal component analysis was carried out using the pooled morphometric data. Negative PC scores were indicative of benthic feeding fish and fish with a positive score were indicative of a pelagic feeding fish. Thus tagged fish were classified as either benthic or pelagic based on their PC score from the morphometric analysis.

6.3.4. Acoustic Position Estimates

To calculate the approximate position of each fish at any given time, the centre of activity (COA; the weighted means number of detections at each receiver) was calculated (Simpfendorfer *et al*., 2002; Hedger *et al*., 2008). To ensure appropriate detection rate and accurate spatial resolution (Simfendorfer *et al*., 2002) an appropriate time bin (Δ_t) was selected following procedures described in Villegas-Rios *et al*., (2013). Six different Δ_t were tested: 10, 20, 30, 40, 50 and 60 minutes. The optimal time bin was considered when the mean number of receivers detecting signals from a single tag (NR) was < 10% between Δ_t however the mean number of detections (ND) remained > 10. The optimal value for this study was Δ_t = 50 min at which NR was 2.08 ± 0.19 and ND was 13.78 ± 1.23 (mean ± SE).

6.3.5. Home Range Analysis

To estimate the extent of the home range, minimum convex polygons (MCP_{100}) were calculated for every COA for each fish, for each month of the study (July, August & September). A second estimate of home range was calculated using fixed kernel utilization distributions (KUDs) based on 95% (home range) and 50% (core range) of positions. An issue facing kernel methods is the appropriate choice of a smoothing parameter or bandwidth selection, (Silverman 1986; Kernohan *et al*., 2001; Vokoun, 2003). Choice of bandwidth is important, as different amounts of smoothing are applied to detections which will result in varying numerical estimates of home range. Therefore, appropriate selection is required to ensure accurate estimates. One method of band selection using least-squares cross validation (LSCV) has been shown to provide the most accurate estimators in kernel analysis (Worton, 1989, Seaman & Powell, 1996), however this method has been shown to result in unrealistic home range estimates elsewhere (Blundell *et al*., 2001). The second most common method for bandwidth selection is reference bandwidth, which has been shown to perform better than other methods of bandwidth selection (Bowman, 1985). Both methods were used in this analysis. The analysis undertaken found LSCV produced excessively large home range estimates which were unrealistic, given the size of the study site. Reference bandwidth also produced some excessively over-smoothed estimates for some fish. To produce more realistic home range estimates a single kernel bandwidth (h) was selected and fixed for KUD estimation. After examination of realistic bandwidths using reference bandwidths (h=href), the average reference bandwidth (h=60) was selected and manually set for each fish. Home range analysis was conducted using the *adehabitat* package (Calenge, 2006) in R statistical software (R Core Team, 2016). As KUD estimates the intensity of habitat use within the

detected range and MCP describes total coverage of an individual's home range MCP_{100}, KUD_{95} and KUD_{50} were all compared between benthic and pelagic *S. trutta* for each month using a linear model approach.

6.3.6. Movement Patterns

The daily movement of each fish was calculated by summing the distance travelled between sequential COA's (Δ_t = 50 min) in the study and divided by each individuals body length to produce average body lengths per 50 mins (BL^{-50min}). The total distance travelled for each fish per month (July, August & September) was also calculated. To calculate daily diel movements, the average distance travelled during particular times of the days was calculated for each hourly period (i.e. 00:00 – 01:00; 16:00 – 17:00 etc) and each hour was assigned to a time bin associated with each time of day category (dawn, day, dusk, night; based on the NOAA sunrise/sunset calculator (NOAA, 2014) during each month. A Shapiro-Wilk test was used to test normality of total distance travelled data, the hypothesis that data were normal failed to be rejected (W = 0.91, P = 0.14) thus total distance travelled was compared using a Welch Two Sample t-test. Fish that exhibited no average hourly movements were considered dead and removed from analysis. Using the average body length for each morphology, body lengths per 50 minutes was calculated into distance travelled (m) per hour (m/hour). As there are multiple measures from each fish, a random effects mixed model methods approach (GLMM) was used to test the effects of average movement rates per hour per month for individuals (Bolker *et al*., 2009). A large number of first order models (full models) were generated and compared with best models being selected with minimum Akaike Information Criterion (AIC). Lowest AIC models sometimes had non-significant independent variables. Final models were generated with non-significant variables dropped. Significant independent variables were removed from final models and compared against models without the significant independent variables (full versus 'null' models) using ANOVA. Movement rates (BL^{-50min}) were used as a continuous response variable, fixed effects included; an individual's length (mm), morphology (benthic or pelagic), month (July, August or September), hour of day (00:00 – 23:00), time of day (Day, Night, Dawn or Dusk) and fish acoustic tag code was used as a random effect.

6.3.7. Residency

The residency of each fish within the fixed array was estimated by calculating a residence index (R_I). The R_I was calculated by dividing the total number of days an individual was detected (DD) by the total number of days of the detection period (TD) (Afonso *et al*., 2008):

$R_I = DD/TD$

Thus each fish R_I fell between 0 (no residency) and 1 (full residency) and was estimated for each month in the study. A Shapiro-Wilk test was used to test normality of residency index data, the hypothesis that data was normal was rejected ($W = 0.74$, $P < 0.001$), thus a Wilcoxon rank sum test was used to measure differences in residency index.

6.4. Results

6.4.1. Fish Details

In total twenty *S. trutta* (13 pelagic – mean length (mm): 226.23 ± 5.59 S.E. and mean weight (g): 124.12 ± 9.6 S.E. and seven benthic – mean length (mm): 226.57 ± 15. 90 S.E. and mean weight (g): 105.67 ± 12.11 S.E.), were individually tagged and released (Table 6.1.). Of the twenty fish tagged, one benthic *S. trutta* and four pelagic *S. trutta* exhibited no movement data between COA's and were presumed mortalities and removed from further analysis. Thus, the following results are based on six benthic and nine pelagic fish (n =15 total). The detection period for tagged fish ranged from 15-92 days (Table 6.1.).

6.4.2. Ecotype Classification

Canonical Variate Analysis (CVA) found that the head shape of tagged benthic and tagged pelagic *S. trutta* was significantly different (Fig. 6.2.) (Procrustes distance = 0.0718, $P < 0.001$; Mahalanobis distance = 3.221, $P < 0.001$).

6.4.3. Home Range Area Estimates

Home range estimates are presented as the MCP_{100} (total home range), KUD_{95} (home range use) and KUD_{50} (core range) (km^2), per month for both pelagic and benthic ecotypes (Table 6.2.). There was no significant difference in total benthic (mean MCP_{100}: 0.256 km^2 ± 0.21 S.D.) or pelagic (mean MCP_{100}: 0.233 km^2 ± 0.21 S.D.) home range between months (ANOVA, $F_{2,30} = 2.8362$, $P = 0.07$) or between ecotypes (ANOVA, $F_{1,30} = 0.21$, $P = 0.65$). There was no significant difference in total benthic (mean KUD_{95}: 0.400 km^2 ± 0.28 S.D.) and pelagic (mean KUD_{95}: 0.389 km^2 ± 0.37 S.D.) KUD_{95} between months (ANOVA, $F_{2,30} = 2.88$, $P = 0.07$), or ecotypes (ANOVA, $F_{1,30} = 0.32$, $P = 0.57$). After removal of a single outlier, there was no significant difference in total benthic (mean KUD_{50}: 0.127 km^2 ± 0.21 S.D.) and pelagic (mean KUD_{50}: 0.060km^2 ± 0.05 S.D) KUD_{50} (core use) between months (ANOVA, $F_{2,29} = 2.81$, $P = 0.07$) or ecotypes (ANOVA, $F_{1,29} = 0.47$, $P = 0.49$). The length of fish did not affect MCP_{100} (ANOVA, $F_{1,30} = 0.71$, $P = 0.4$), KUD_{95} (ANOVA, $F_{1,30} = 3.18$, $P = 0.08$) or KUD_{50} (ANOVA, $F_{1,29} = 3.57$, $P = 0.06$).

6.4.4. Habitat Use

There was extensive overlap of core area habitat use between benthic and pelagic fish (Appendix I). Benthics were consistently found near the shoreline (shallow, cobble habitat) of Lough Finn in the west basin, and pelagic fish were regularly found moving in areas of deep water (Appendix I).

6.4.5. Residency

Four *S. trutta* immediately left the array following tagging; two pelagic and two benthic. All four fish returned in August and remained resident in the array throughout September. A Wilcoxon rank sum test revealed no difference in residency index between ecotypes ($W = 35$, $P = 0.42$)

6.4.6. Movement Patterns

A Welch Two Sample t-test indicated (body lengths per 50 mins (BL^{-50min}) between benthic and pelagic *S. trutta* were not statistically significant ($t = -0.88$, $P = 0.39$), pelagic *S. trutta* movement was approximately 6.3m/h and benthic *S. trutta* approximately 7.2m/h.

6.4.7. Diel Movements

The minimal adequate linear mixed model through lowest AIC and removal of non-significant interaction terms for *S. trutta* movement revealed a significant effect of month on average daily fish movement (BL^{-50min}) ($\chi^2 = 92.45$, $df = 2$, $P < 0.001$). In July, there was no significant difference in movement between ecotypes ($\chi^2 = 0.03$, $df = 1$, $P = 0.86$). Time of day (Night, Day, Dawn and Dusk) however, had a significant effect on movement in both fish types ($\chi^2=13.41$, df=2, $P = 0.001$), with both ecotypes being less active throughout the day (mean benthic 11.17m/h; pelagic 11.88m/h) and more active during the night (benthic 14.4m/h; pelagic 15.18m/h), immediately before dawn (Fig. 6.3.). In August there was no significant difference in movement between ecotypes ($\chi^2 = 0.49$, $df = 1$, $P = 0.48$). Time of day (Night, Day, Dawn and Dusk) however, was significantly affecting movement in both fish types ($\chi^2 = 7.03$, $df = 2$, $P = 0.001$), with fish more active throughout the day (mean benthic 10.75m/h; mean pelagic 8.52m/h) and less active during the night (mean benthic 9.79m/h; mean pelagic 7.68 m/h) (Fig. 6.3.). There was no significant difference in movement between ecotypes throughout September ($\chi^2 = 3.68$, df = 1, $P = 0.05$), time of day did affect fish movement ($\chi^2 = 7.30$, $df = 2$, $P = 0.02$) with fish more active throughout the day (benthic 15.53m/h; pelagic 7.96m/h) and less active during the night (benthic 12.15m/h; pelagic 7.83m/h). Although no statistical significance was found between ecotypes, benthic *S. trutta* movement peaked around midday throughout September (Fig. 6.3.).

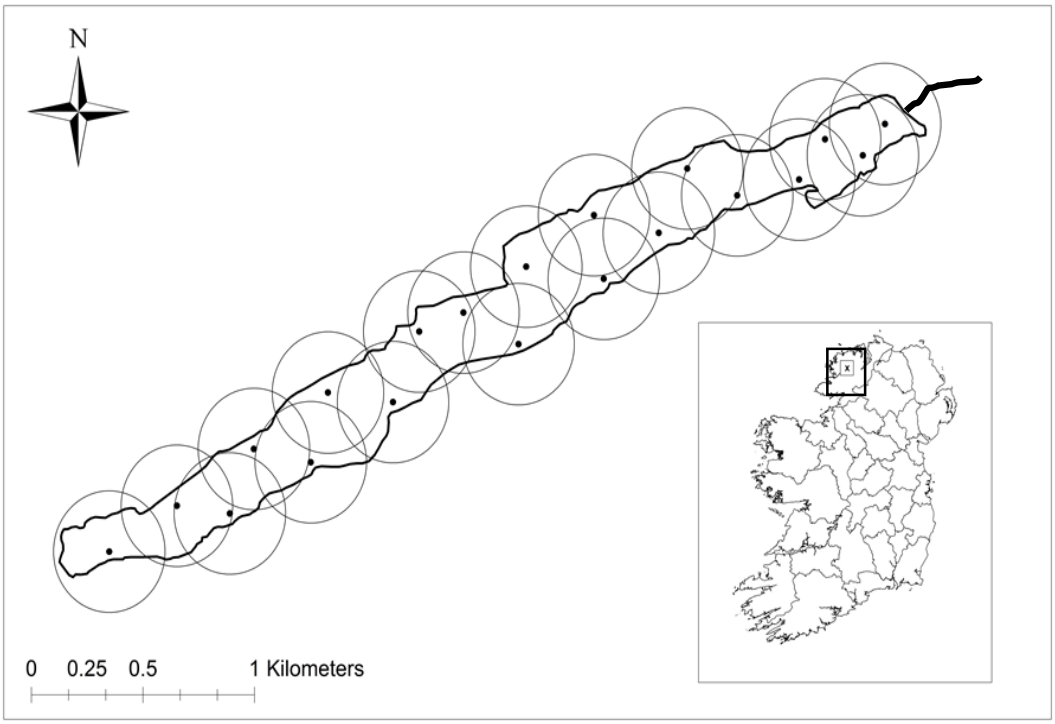


Figure. 6.1. Lough Finn and river Finn outflow stream, receiver positons (black dots) and omnidirectional detection range from acoustic listening station (black circles).

Table 6.1. Characteristics of the 20 individuals tagged, including acoustic tag code (Fish ID), release date, Fork Length (mm), weight (g), detection span (days), ecotype and Residency Index (R_I)

Fish ID	Release Date	Fork Length (mm)	Weight (g)	Detection span (days)	Ecotype	Residency (R_I)
2301	02/07/2013	239	144.1	91	Benthic	1
2304	02/07/2013	185	89.6	87	Benthic	0.96
2305	02/07/2013	195	82	91	Benthic	1
2307	02/07/2013	298	77	80	Benthic	0.87
2314	02/07/2013	197	86	15	Benthic	0.17
2319	02/07/2013	242	147	23	Benthic	0.25
2321	02/07/2013	230	114	34	Benthic	0.37
2306	02/07/2013	228	122.4	80	Pelagic	0.88
2308	01/07/2013	207	93	78	Pelagic	0.85
2309	02/07/2013	230	126	36	Pelagic	0.39
2310	02/07/2013	219	196	84	Pelagic	0.92
2311	02/07/2013	178	54	91	Pelagic	1
2312	02/07/2013	249	154	90	Pelagic	0.99
2313	01/07/2013	229	114.2	92	Pelagic	1
2317	01/07/2013	219	118	92	Pelagic	1
2318	02/07/2013	236	129.5	26	Pelagic	0.29
2324	01/07/2013	218	92.5	92	Pelagic	1
2328	02/07/2013	245	137	88	Pelagic	0.97
2331	02/07/2013	225	120	78	Pelagic	0.86
2338	02/07/2013	258	157	36	Pelagic	0.40

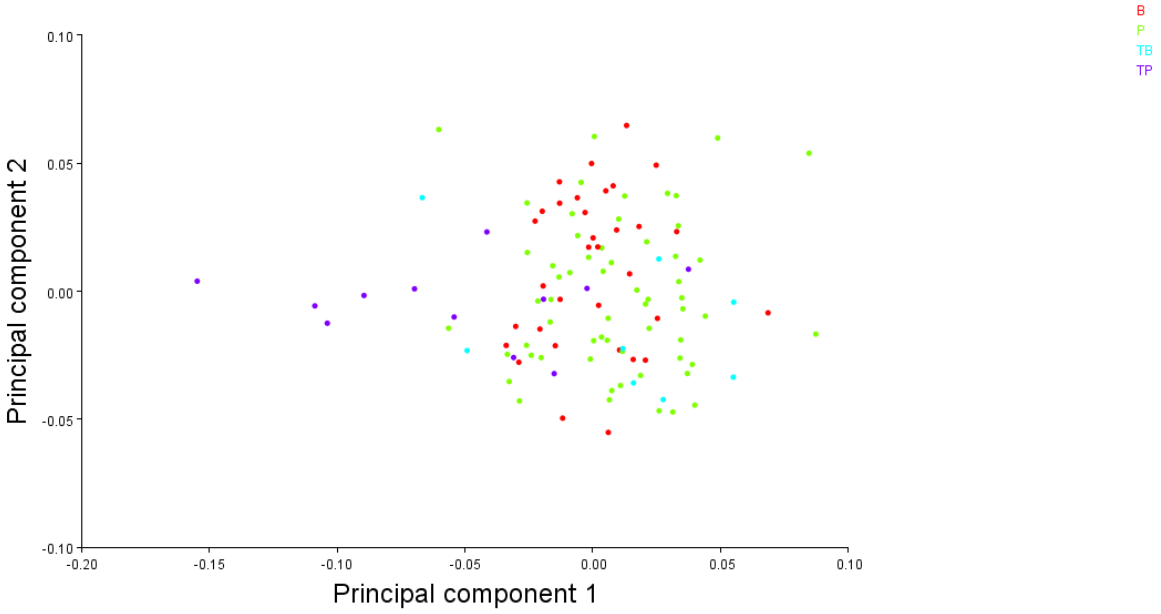


Figure. 6.2. Canonical Variate Analysis of 117 Lough Finn *S. trutta* plus 10 tagged pelagic and 10 tagged benthic *S. trutta*. Purple dots represent tagged pelagics, blue dots represent tagged benthics, green dots represent non-tagged pelagics and red dots represent non-tagged benthics.

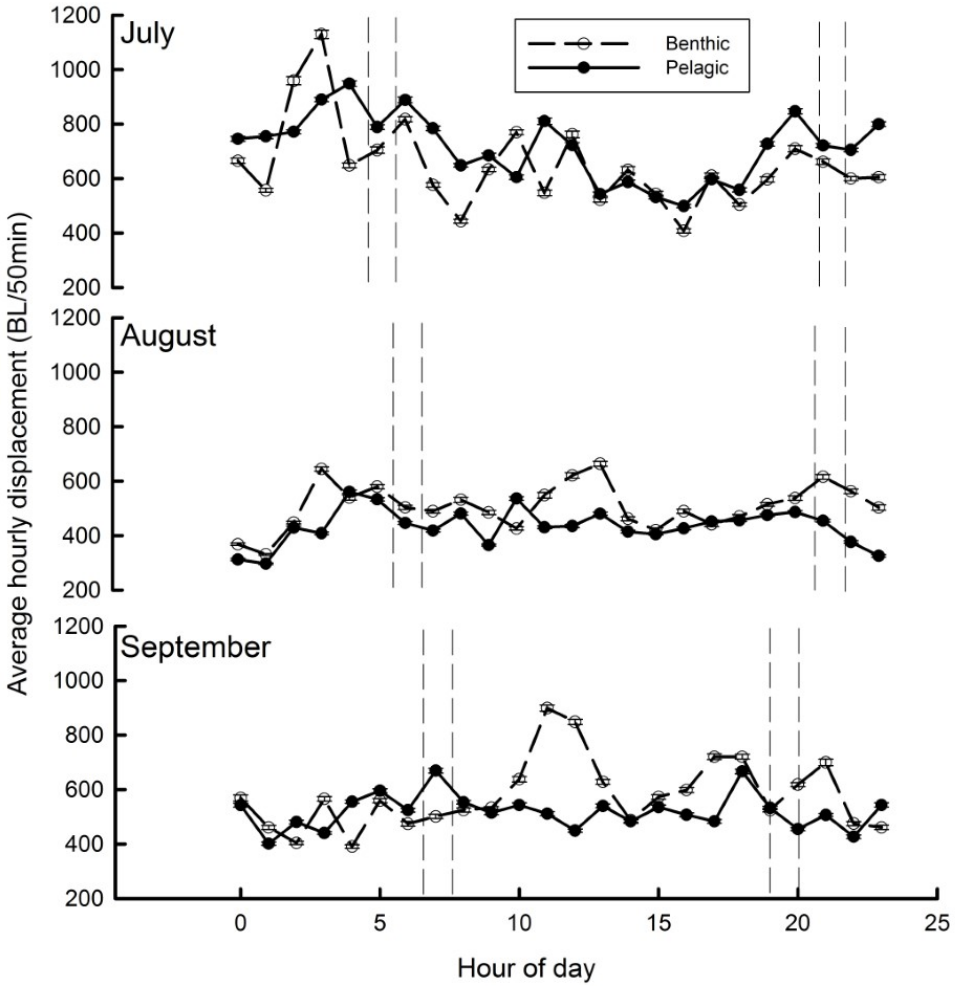


Figure. 6.3. The average movement rate (BL^{-50min}) per hour (facet by month) for benthic (B=white circles) and pelagic (P=black circle) individuals. Crepuscular periods are represented by light shading (range = min and max sunrise/sunset for each month, NOAA 2014).

Table 6.2. Mean KUD_{95}, KUD_{50} and MCP 100 per month and overall mean for the duration of tagging period. All units are km^2.

Month	Benthic KUD_{50}	Benthic KUD_{95}	Benthic MCP 100	Pelagic KUD_{50}	Pelagic KUD_{95}	Pelagic MCP 100
July	0.102	0.539	0.367	0.089	0.645	0.374
August	0.196	0.284	0.147	0.043	0.284	0.186
September	0.075	0.434	0.300	0.053	0.283	0.165
Overall mean	0.124	0.419	0.271	0.062	0.404	0.242
(S.E)	0.036	0.074	0.065	0.014	0.121	0.066

6.5. DISCUSSION

I found no difference in home range (MCP_{100} or KUD_{95}) or core range (KUD_{50}) between benthic and pelagic ecotypes. This is surprising as pelagic ecotypes have been found to use larger home ranges than benthic counterparts in other species (Amundsen *et al*., 2008; Hawley *et al*., 2016). Given the small size of Lough Finn, *S. trutta* found there may utilize similar home ranges than would be found in a larger lake system. Although no difference in core range size was found, benthic trout tended to inhabit the south western part of the lake, which was shallower with more pebble and cobble substrate compared with pelagic trout which were commonly found using the north eastern part of the lake in areas of deep water (Appendix I). Overlap did occur between benthic and pelagic, however this data may reflect the alternative foraging behaviour of each type.

Residency of *S. trutta* was not consistent throughout the study ranging from 0.17 to 0.99 residency (excluding full residency individuals who were removed from analysis) (Table 1). Four trout immediately left the acoustic array following tagging (two benthic and two pelagic) and were last detected at the outflow receiver suggesting entrance to the river. Given that an equal amount of fish from both ecotypes left the array, the reasons for this behaviour is unclear and may have been a response to the tagging procedure, although surgical implantation appears to have a minimal effect on behaviour in salmonids (Adams *et al*., 1998; Miller *et al*., 2013; Hondorp *et al*., 2015). Importantly, the four fish returned and their subsequent movement patterns were recorded. As rivers are critical to juvenile *S. trutta* development (Armstrong *et al*., 2003), the observation of mature lacustrine *S. trutta* utilising limnetic systems, out with the spawning period (October – December), perhaps as a response to stress, is surprising.

There was no significant difference in total movement between benthic and pelagic *S. trutta*, which is contrary to our prediction that pelagic *S. trutta* would be more active. Studies of other pelagic and planktivorous modes of foraging, found pelagic fish to be much more active than benthic/ profundal counterparts (Amundsen *et al*., 2008; Hawley *et al*., 2016). Amundsen *et al*., (2008) demonstrate pelagic *S. alpinus* had more of a generalist feeding strategy compared with profundal (benthic) *S. alpinus*, which had a narrower niche width, thus pelagic fish utilised larger areas of habitat and were subsequently more active.

Diel movement in *S. trutta* is often reported as nocturnal with highest activity recorded during hours of darkness, particularly during low temperatures (Heggenes *et al*., 1993; Young, 1999) however diurnal rhythms have also been reported (Roussel & Bardonnet, 1995). In July, both ecotypes exhibited a nocturnal diel rhythm. In August and September however, this diel movement switched from nocturnal to diurnal with both ecotypes exhibiting highest movement during daylight hours (Fig.6.2.). A fish's ability to detect prey increases with light intensity (Fraser & Metcalfe, 1997) however so does the risk of predation (Metcalfe *et al*., 1999). Thus, a fish's motivation to capture prey during riskier time periods may be due to an increase in nutritional needs such as an increase in temperature (Fraser *et al*., 1993). This study began in mid-summer (July) and progressed into late summer (September). During these months there is a clear shift from nocturnal to diurnal movement in both ecotypes (Fig. 6.2.). This shift in diel movement suggests a shift in foraging behaviour in all *S. trutta* tagged in this study. Interestingly, benthic *S. trutta* activity peaked at midday in September, something that has been recorded in riverine *S. trutta* (Elliot, 1970) and Cutthroat trout *Oncorhynchus clarki* (Young *et al*., 1997) mainly due to peaks in drifting terrestrial and aerial invertebrates. In a lacustrine system however, this appears unlikely and is probably not the contributing factor to the observed midday peak in activity in benthic *S. trutta*. One potential explanation is the reduction in daylight in September, which may force benthic *S. trutta* to forage in optimum light conditions to maximise foraging success and ensure adequate fat accumulation for the approaching spawning and over-winter season. There is also evidence to support the emergence of freshwater invertebrates in this region during September (Barry pers comm.) which may encourage benthic ecotypes to become more active.

The present study indicates that the movement patterns of lacustrine *S. trutta* ecotypes is not as clear as movement observed in other species ecotypes. Both ecotypes in this study responded similarly to temporal changes and both exhibited flexible diel cycles, highlighting the plasticity of *S. trutta* in response to their environment. Examination of fine-scale movements in ecotypes is an important aspect of ecological

and evolutionary biology. Furthermore, this information can be used for effective fisheries management strategies and thus warrants further investigation.

CHAPTER 7.

GENERAL DISCUSSION

7.1. THESIS AIMS

The goals of the research presented in this thesis were to test long held assumptions about ecological parameters and growth patterns associated with the elusive ferox trout. I also investigated the physiological, behavioural and ecological factors that may contribute to the recruitment of ferox trout populations and their potential importance on broader evolutionary issues such as incipient speciation. I tested long held hypotheses on environmental characteristics associated with wild ferox trout populations by examining abiotic and biotic data associated with known ferox trout habitats using robust statistical analyses (chapter 2). I also tested conventional models of ferox trout growth by back-calculation of ferox trout and sympatric brown trout scales followed by construction and comparison of growth curves (chapter 3). For this thesis I acquired spawning ferox trout and successfully hatched eggs, which to my knowledge is the first time this has been done with ferox trout. As a result, I focused heavily on investigating differences between juvenile ferox trout and brown trout in an attempt to identify biological mechanisms during early ontogeny that may contribute to the divergence in ferox trout and brown trout we observe in sympatric populations. I examined maternal provisioning, embryonic development, survival rate, hybrid viability, head shape morphology, metabolism, lipid deposition (chapter 4) and dominance behaviour (chapter 5). Finally, I investigated the spatial movement of two distinct brown trout ecotypes using acoustic telemetry in a post-glacial lake (chapter 6).

In this chapter I will briefly discuss the current literature and how the findings of this thesis contribute to our overall understanding of ferox trout. Lastly I discuss the limitations of my research, the importance of ferox trout research and the future directions based on these findings.

7.2. CURRENT LITERATURE

7.2.1. FEROX TROUT

The current literature on ferox trout globally is sparse. These elusive fish are notoriously hard to sample (Duguid *et al*., 2006) and identification is complicated by taxonomic debate (Ferguson, 2004). The literature that does exist however describes an incredibly rare (Mangel & Abraham, 2001; Duguid *et al*., 2006) freshwater predator (Grey, 2001) that can delay maturation (Campbell 1971; Mangel & Abrahams, 2001), grow to

enormous proportions (Campbell, 1979; Greer, 1995) and has an extended life span (Mangel & Abrahams, 2001). These qualities alone make ferox trout a source of extreme interest however, when accompanied with extensive genetic (Ferguson & Mason, 1981) and phenotypic (Cawdery & Ferguson, 1988) variation relevant to both evolutionists and conservations, interest increases exponentially.

Genetic investigation of ferox trout carried out extensively in Ireland (predominately Lough Melvin) (Ferguson & Mason, 1981; Hamilton *et al*., 1989; Ferguson, 1989; Ferguson & Taggart, 1991; Hynes *et al*., 1996) and Scotland (Duguid *et al*., 2006) found ferox trout represent an ancestral lineage of *S. trutta*, and ferox trout inhabiting Loch Melvin, Awe and Laggan are more closely related than *S. trutta* in the same lake. These important discoveries prompted the reclassification of ferox trout as *S. ferox* by the IUCN, alongside the benthic gillaroo *S. stomachicus* and the pelagic sonaghen *S. nigripinnis* (Freyhof & Kottelat, 2008). Importantly as gillaroo and songahen were classified on the ICUN red list as 'Vulnerable', ferox trout were categorised as 'Data deficient' a reflection on the lack of substantial published literature.

7.1.2. Fish In Post-Glacial Lakes

Fish inhabiting post-glacial lakes represent some of the best examples of trophic specialisation in any taxa (Schluter, 2000). The invasion of anadromous ancestral fish of lakes immediately following deglaciation allowed invading fish to exploit numerous replicated depauperate environments. These young ecosystems provide all the characteristics necessary to enable adaptive radiation of phenotypically plastic species to occur and has resulted in the appearance of multiple resource polymorphism across a number of freshwater fish species including: Three-spine sticklebacks *Gasterosteus* spp, Rainbow smelt *Osmerus mordax*, Lake Whitefish *Coregonus* spp, Arctic charr *Salvelinus alpinus* and Brown trout *Salmo trutta*. The examination of genetic, ecological, physiological and behavioural variation within, and between, species have been of huge evolutionary significance. Most staggering is the relative rate of sympatric divergence believed to have occurred within the last 15 000 years. Thus, freshwater fish species inhabiting post-glacial lakes have and continue to be important study systems for the potential early mechanisms of divergence and ecological speciation

Therefore, by focusing on ferox trout as the main target species for this research I have been able to tackle important research topics pertinent to management, conservation and evolutionary biology.

7.3. Furthering Our Understanding

Perhaps a full understanding of the elusive ferox trout will never be completely possible, however the results of this thesis provide substantial evidence that environmental, physiological and behavioural characteristics play a significant role in the production and maintenance of ferox trout. I also provide evidence of spatial and temporal differences found between *S. trutta* exploiting benthic and pelagic resources, which may be an important driver of incipient speciation.

In chapter 2 I discovered abiotic and biotic characteristics, long associated with ferox trout populations, had strong predictive power on extant ferox trout populations. Through a series of binomial logistic regressions, I found lake depth (m), lake surface area (km^2), lake catchment (km^2) and the presence of Arctic charr *S. alpinus* were all positively correlated with the presence of ferox trout. Furthermore, I produced a modern estimate of 192 lakes which have published evidence of producing ferox trout. Using this information, I constructed a probability model based on the most readily available environmental parameter, lake area. The probability model estimated approximately 366 lakes in Scotland are large enough to support a ferox trout population. This comparatively low number combined with the rarity of individual ferox trout within a lake system (Duguid *et al*., 2006) highlights the vulnerability of ferox trout. It also suggests their distribution is determined by environmental characteristics and not genetics alone.

In chapter 3 I revealed ferox trout populations do not always follow conventional models of growth i.e. slow growth followed by rapid growth after a switch to piscivory. In Loch Rannoch I found ferox trout did subscribe to this conventional model of growth and had significantly different growth rates to sympatric brown trout post-switching, but not pre-switching. In Loch Awe ferox trout exhibited distinct growth trajectories from sympatric *S. trutta* from birth and did not exhibit a measurable 'switch' to piscivory. In Loch na Seagla no recognisable difference in growth was found between ferox trout and sympatric *S. trutta.* All ferox trout were found to be significantly older than brown trout, regardless of growth trajectory. Thus, the results of chapter 3 demonstrate that there are multiple ontogenetic growth pathways to achieving piscivory in *S. trutta* and adopting a piscivorous diet may be a major contributing factor to expansion of life span in ferox trout.

In chapter 4 I found offspring from parents exhibiting alternate life histories differed significantly in a range of traits. I found ferox trout females produced larger eggs than sympatric *S. trutta*. I also found offspring of ferox trout had larger yolk sacs at emergence, higher rates of survival, lower body lipid content and significantly different head shape

compared to offspring from benthivorous brown trout. I found no differences in metabolism or embryonic developmental pace between offspring from ferox trout and benthivorous brown trout. These results provide strong evidence that the life-history adopted by the parents, is likely to influence the life history trajectory of their offspring. Interestingly hybrid crosses between ferox trout and benthivorous trout consistently fell between the range of measurements although suffered high mortality suggesting a level of hybrid inviability.

In chapter 5 I determined parental type had a significant effect on dominance behaviour of offspring. Juvenile ferox trout were more aggressive, held better spatial positions, exhibited bright colouration (associated with dominance) and acquired more food than size matched juveniles from benthivorous parents. These results suggest that the life history of parents directly influences the dominance behaviour of offspring. This behavioural mechanism, if replicated in the wild, would almost certainly infer greater feeding and thus growth in ferox trout, allowing them to exploit larger prey items, such as fish, at a younger age.

Lastly in Chapter 6, I found no differences in overall home range, core range and movement between *S. trutta* expressing benthic and pelagic phenotypes in an oligotrophic lake. Although no inferences about foraging behaviour can be inferred statistically for this research, both ecotypes did respond similarly to temporal changes; changing diurnal rhythms from nocturnal to diurnal across a three-month period. Interestingly, movement of benthic ecotypes peaked during midday in September, which may coincide with emerging freshwater invertebrate larvae. These results suggest environmental parameters still heavily modulate movement patterns in *S. trutta* in this lake.

7.4. Limitations Of My Studies

Like all publications preceding this thesis, the biggest limitation to these studies was ferox trout sample sizes (Ferguson & Taggart, 1991; Duguid *et al*., 2006). While my results corroborate with much of the previous literature and is likely important for other ferox trout populations, the lack of replication at the family level in my studies restricts the ability to make extensive generalisations about ferox trout physiology, ecology and behaviour out-with the catchment used in this study. Ideally, a repeat of the common garden experiment (chapter 3) using a substantial amount of ferox trout families from different catchments would be extremely useful, however likely very difficult, given the rarity of ferox trout as well as the logistical constraints of such a system including the long-term housing and husbandry of offspring. The second limitation of this study is the use of tags with multiple

functions. Although movement data is interesting and extremely useful for upstream and downstream migrations of salmonids (Rustadbakken *et al*., 2004; Newton *et al*., 2016), the information gained from lacustrine studies can be more limited. Ideally, the use of depth tags in our telemetry study would have provided finer resolution and potentially separation of the pelagic and benthic ecotypes, although financial implications would make replication of such a study relatively expensive.

7.5. Importance Of Ferox Trout Research

Debate about ferox trout dates back to Berkenhout (1789) and continues to this day. Yet fundamental questions about their basic biology are still poorly understood. The little research that has been conducted on ferox trout has been disproportionately useful to both evolutionary biology and conservation biology, however.

7.5.1. Evolutionary Biology

Subjected to extensive genetic investigation in Ireland (Ferguson & Mason, 1981; Hamilton *et al*., 1989; Ferguson, 1989; Ferguson & Taggart, 1991; Hynes *et al*., 1996) and Scotland (Duguid *et al*., 2006), ferox trout research provided a platform for subsequent research on the phylogeographic structure of *S. trutta* and thus provides information on the ancestral lineages invading post-glacial lakes across north-western Europe after the last glaciations - the complexity of which is still being investigated (McKeown *et al*., 2010). Not only do ferox trout represent an ancestral lineage of trout, they co-exist with at least one other lineage in sympatry. Understanding the early mechanisms following the invasion of a species into a depauperate habitat is fundamental to evolutionary biology. In addition to this how co-habiting polymorphisms maintain reproductive isolation, distinct phenotypes and unique ecology in the absence of geographical barriers is critical to our understanding of incipient species formation and preservation. Thus, from an evolutionary perspective, the understudied ferox trout represents a model species as significant as Three-spine stickleback, Lake whitefish and Arctic charr.

7.5.2. Conservation Biology

A consequence of the discovery of distinct *S. trutta* lineages in Lough Melvin, Loch Awe and Loch Laggan (Ferguson & Mason, 1981; Ferguson & Taggart, 1991; Duguid *et al*., 2006) was a review on the effectiveness of conventional conservation strategies in protecting unique biodiversity. The review by Ferguson (2004) on recognition of biodiversity and conservation units highlighted the inefficiency of taxonomic classification in protecting unique wildlife. Ferguson (2004) discounts species designation as an adequate form of

protection for local biodiversity and proposes community-led strategies should be adopted in conjunction with the protection afforded from species designation. Ferguson (2004) also highlights potential dangers facing ferox trout - risks shared by all freshwater fish species of Ireland and Britain - such as the introduction of non-native species, namely the zebra mussel *Dreissena polymorpha* and rudd *Scardinius erythropthalmus*. A comparable example can be found in Loch Lomond where the endemic Powan *Coregonus clupeoides* population is at risk of decline following the introduction of a non-native fish, the Eurasion ruffe *Gymnocephalus cernua* (Adams & Maitland, 1998). The research on ferox trout, since Ferguson & Mason (1981), also serves as an example highlighting the lengthy process involved in evidence based taxonomic classification. In 2008, ferox trout gillaroo and songahen in Lough Melvin were formally recognised by the IUCN as distinct species, 27 years after reinstating their previous taxonomic classification was proposed (Ferguson & Mason, 1981). The significance of publicising the conservation issues facing ferox trout on broader conservation concerns cannot be understated. From an ecological viewpoint, ferox trout undoubtedly play a significant role as apex predators (Grey, 2001), contributing to community structuring and overall ecosystem health (Estes *et al*., 2011). Their longevity (Mangel & Abrahams, 2001) and sheer size also indicate that their presence alone indicates a well-functioning ecosystem.

7.5.3. ECOMONIC VALUE

As large, elusive and predatory fish, the ferox trout unsurprisingly attracts the attention of anglers. Given their rarity and unusual lake movements deep in the water column (Thorne *et al*., 2016), capture of ferox trout requires specialised trolling techniques from slow moving boats (Greer, 1995). The cost of appropriate equipment, boat hire/ fuel and rod licences ensure ferox angling is an expensive pastime. Since the first extensive review on ferox trout by Campbell (1979), the interest in ferox trout has increased. The formation of the Ferox85 group and the publication of Greer (1995) provided accessible data and information of interest to both scientists and anglers. The local economic benefit provided through ferox trout angling has been questioned (Greer, 1995) and is yet to be quantified, however it may be significant to isolated rural communities with little or no real tourism. Research, and perhaps more importantly the communication of research, assessable to all, is therefore imperative to help promote, conserve and further our understanding of this potentially vulnerable fish.

7.6. Potential Future Directions

Throughout the latter half of the 20th century, the progress made in developing powerful genetic tools used for analysis was evident across all fields of biology. The pace of such developments has accelerated, with genome wide analysis now available for some species (Jaillon *et al*., 2004; St. John *et al*., 2012). If adequate samples could be obtained across Scotland and Ireland, a nationwide investigation of brown trout diversity, similar to that conducted in Lough Melvin would undoubtedly reveal more incredible diversity. Personally, I think the geographic split of ferox trout on the west coast of Scotland and Ireland (Awe, Laggan, Melvin), versus a less clear scenario elsewhere (Rannoch, na Selaga), suggests ancestral ferox trout have a limited distribution across Scotland. Identifying such a boundaries would be invaluable to further our understanding of ferox trout distribution and conservation.

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APPENDIX I

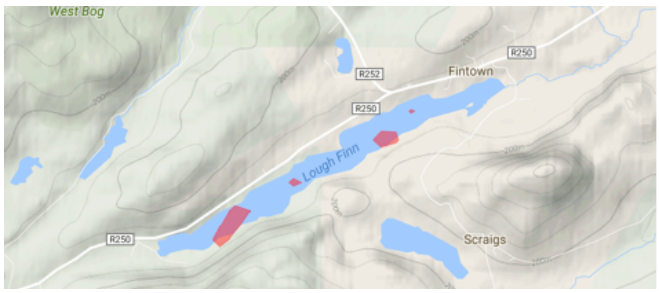
A.1. MAPS

KUD_{50} (core range) per month for each fish used in analysis. *Note fish with no movement data for study duration or for specific months are excluded.

Fish 2301

August

Fish 2307



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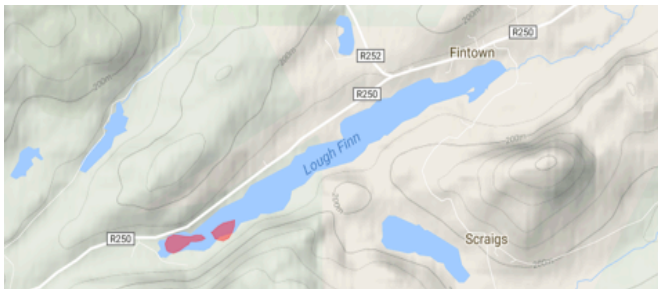

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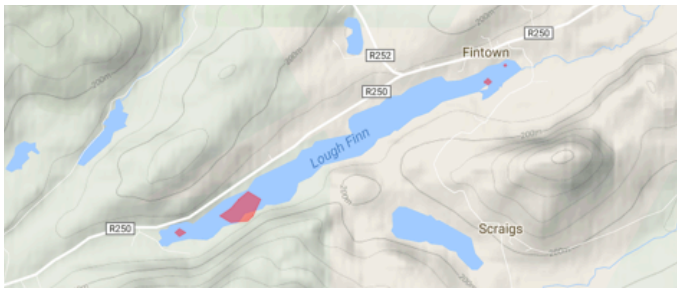

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Fish 2312

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Fish 2313

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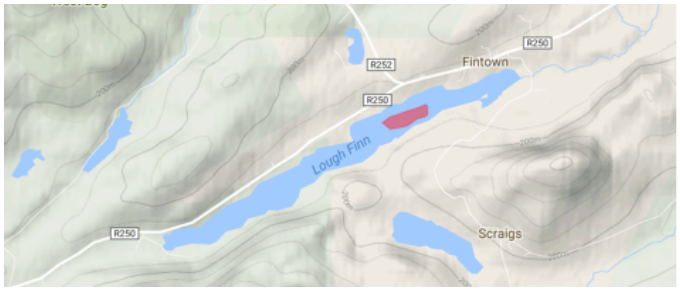
Fish 2314



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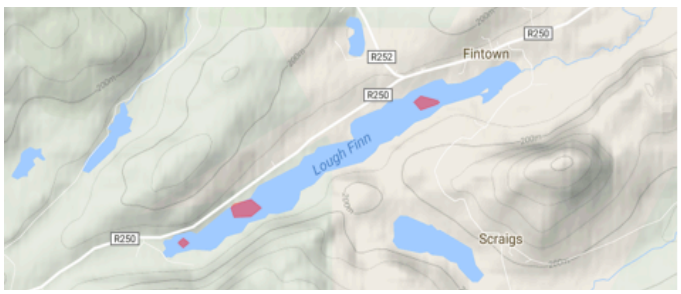

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Fish 2324

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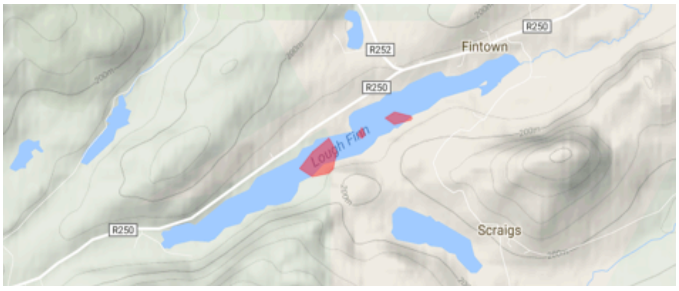
Fish 2328



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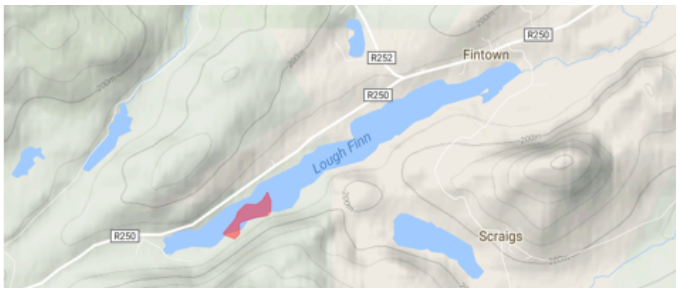
Fish 2331



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APPENDIX II
PRIOR RESOURCE AVAILABILITY AND LIFE HISTORY ORIGIN AFFECT COMPETITIVE BEHAVIOUR IN TERRITORIAL DISPUTES

*Note: This chapter has been published in the journal Behavioral Ecology.

A.1. INTRODUCTION

Dominance hierarchies generally result in dominant individuals gaining preferential access to food, shelter or mates. Differences in dominance-related traits (e.g. aggression, food acquisition, competitive ability etc.) have been linked to variation in growth (Vøllestad & Quinn 2003; Adriaenssens & Johnsson 2011) and life history strategies (Biro & Stamps 2008; Chapman *et al*. 2011) but may be dependent upon condition. For example Van Leeuwen *et al*. (2011) demonstrated that dominant individuals experienced higher growth rates than subordinates when food was abundant but had lower growth rates than subordinates at low food as dominant individuals, being larger, became more constrained by the food availability in their habitat.

Maximising energy intake (food consumption) without also increasing energy expenditure (through costs of foraging, defending territories and movement) is a problem faced by many organisms. If habitats differ predictably in their productivity, this net rate of energy intake is likely to have important implications as to whether it is more profitable for an individual to remain in a locality (resident approach) or leave (migratory approach; Gross *et al*. 1988). There will be individual variation in the net rates of energy intake in a given habitat, and as a result the trade-off between potential net energy intake as a resident or a migrant also differs between individuals. Partial migration, in which some individuals of a population migrate and others remain sedentary is a phenomenon that occurs across a wide range of taxa (see reviews in: Chapman *et al*. 2011; 2012; Dodson *et al*. 2013). The commonest form of this intraspecific variation in movement patterns is non-breeding partial migration (*sensu* Chapman *et al*. 2011), where migrants and residents breed sympatrically but forage in very different habitats. There have been many hypothesized explanations for this category of migration, including intraspecific competition for limited food resources, predation risk trade-offs and intraspecific niche diversity (see Chapman *et al*. 2011).

However, there is a lack of hard evidence of the factors that pre-dispose particular individuals to one or the other strategy. Those that by chance have the advantage of greater access to resources may be less liable to migrate (Sandell & Smith 1991). Similarly,

residency may be more likely in those encountering a low local density of competitors (as has been demonstrated experimentally in red-spotted newts by Grayson & Wilbur (2009), or those experiencing a higher food supply (as in salmonid fish: Olsson & Greenberg 2004; Olsson *et al*. 2006; Wysujack *et al*. 2009). Body size may also affect the selection pressures for/against migration, since larger individuals may generally have less to gain from migration (Dodson *et al*. 2013, though see Brodersen *et al*, 2008 for an exception) since they usually have the advantages of a higher competitive ability and/or lower risk of starvation and predation (Chapman *et al.* 2011).

One area which has received a considerable amount of attention in explaining patterns of partial migration, mainly in birds, has focused on the role of dominance-related traits and body size (Gauthereaux 1982; Nilsson *et al*. 2008). More dominant individuals tend to outcompete subordinates for limited food and breeding resources, which in turn forces subordinates to migrate in search of more profitable environments (Gauthreaux 1982). While this hypothesis has been supported in several studies (Lundberg 1985, Nilsson *et al*., 2008) it has been rejected by others (Rogers *et al*., 1989; Boyle, 2008), indicating that the role of dominance status (and hence often body size) in explaining patterns of life history diversity may be dependent on the particular environmental conditions that are experienced at the time and whether or not an individual's migratory tendency is fixed (i.e. determined by its parents through genetic or parental effects, so that offspring of migrants are themselves migratory), as demonstrated by Berthold (1988) and Berthold & Pulido (1994) for migratory tendency and migration distance in the Blackcap, or flexible (condition-dependent; Brodersen *et al*., 2008).

A well-documented example of partial migration is the brown trout, a polymorphic species that adopts a continuum of life history strategies, with the two most common being freshwater-resident and anadromous migrant (i.e. born in freshwater but growing at sea, before returning to fresh water to spawn). The two ecotypes can occur in sympatry, possibly derived from a single gene pool, with anadromous and freshwater-resident adults having the ability to interbreed and both being able to produce offspring apparently capable of adopting either life history, depending on food availability (Olsson et al. 2006; Wysujack et al. 2009; O'Neal & Stanford 2011), although the probability of adopting either life history may vary depending on parentage (as demonstrated for a closely related species the rainbow trout (*Oncorhynchus mykiss*; Nichols et al. 2008).

While it is likely that genetics interacts with growth history, current body size and physiological condition to determine whether or not the animal migrates, there may also be a role for inherent differences in dominance-related traits independent of body size. Soon after emergence from the nest, brown trout fry rapidly establish dominance hierarchies that are temporally stable (Johnsson & Forser 2002; Jonsson & Jonsson 2010) and engage in intra- and inter-specific competition for preferred feeding territories (Lahti et al. 2002; Klemetsen et al. 2003). It is generally accepted that dominance is advantageous since it gives preferential access to food (Alanära & Brännas 1996) and so facilitates increased growth (Klemetsen et al. 2003), including in the wild (Höjesjö et al. 2002). The degree to which an individual competes for a territory may also depend on its previous experience. In brown trout fry it has been shown that both previous rearing density (Sundström et al. 2003) and habitat preferences (Johnsson et al. 2000) influence territorial competitiveness, which may also be influenced by the fish's nutritional state (Johnsson et al. 1996).

Therefore given that one of the drivers for migration is the relative rates of resource acquisition in different habitats that is likely dependent on traits associated with dominance of the individual we test 1) whether size-matched juvenile offspring of freshwater resident and anadromous brown trout differ in dominance related traits (food acquisition, spatial position, colour and aggression) in dyadic contests when competing for feeding territories in a semi-natural stream channel and 2) whether differences in these dominance related traits depends on the level of food availability that individuals have experienced earlier in life. Differences in relative dominance traits of offspring from alternative life histories would provide evidence for parental effects (genetic and/or non-genetic) as a potential mechanism perpetuating the maintenance of alternative life histories in partially migrating populations, whereas an effect of prior food availability would indicate that dominance related traits could be influenced by non-genetic differences in the quality of their early environment.

A.2. METHODS

A.2.1. BROODSTOCK COLLECTION

Twenty-four mature freshwater-resident (12 male and 12 female) and 14 anadromous (7 male and 7 female) brown trout were captured using electrofishing on 11 and 23 October 2013 from two neighbouring sub-tributaries of the River Tweed, Scotland. Freshwater-resident trout were collected from above an impassable dam on the Whiteadder River (55° 88'N, 2°57'W) while the anadromous trout were collected from the College Burn (55° 77'N, 2°18'W). Adult fish were classified as freshwater-resident or anadromous based on size and

coloration (Eek & Bohlin 1997): freshwater-resident fish were smaller and dark brown in color with red spots, while anadromous fish were larger and silvery-grey in color with black spots. Both ecotypes were transported to the Belhaven Trout Company, Scotland, where they were held separately in two round 1530 L aluminum tanks supplied with 8.1 ± 0.4 °C (mean±SD) well water under ambient photoperiod and assessed every three days for ripeness.

Ripe fish were anaesthetised, blotted dry, and their eggs or sperm extruded by abdominal massage. Eggs were fertilised with sperm from a haphazardly-chosen male of the same life history origin to create 12 full sibling freshwater-resident families and 7 full sibling anadromous families. Freshwater-resident and anadromous fish were artificially spawned from 3 November - 29 November and 17 November - 4 December 2013 respectively.

A.2.2. Egg Rearing, Hatching And Fish Husbandry

Each family of eggs was housed separately in a plastic mesh egg basket, placed in one of two (1m X 3m X 0.4m) rearing troughs supplied with well water and covered with dark plastic sheeting to ensure eggs were in complete darkness. Water temperature during incubation was 8.1 ± 0.4 °C and was recorded daily along with any dead eggs which were carefully removed.

Eggs were checked daily for hatching; those from freshwater-resident and anadromous fish hatched from 19 December 2013 - 17 January 2014 and 30 December 2013 - 24 January 2014 respectively. Once eggs began to hatch, the newly emerged offspring (alevins) were separated from the remaining eggs and gently placed into a small mesh basket (one per family) located in the same two troughs as the egg baskets.

On 31 January 2014 alevins (i.e. hatched embryos still dependent on the yolk sac for nutrition) were transported to the Scottish Centre for Ecology and the Natural Environment, Scotland. Families were housed separately in 15 L (50cm X 30cm X 15cm) clear plastic aquaria on a partial recirculation system at a constant temperature of 9.2 ± 0.2 °C (mean±SD) and simulated ambient photoperiod. The aquaria each contained a single air stone and were supplied with water pumped directly from Loch Lomond, which was first treated with an ozone generator (Sander S1000, Germany) before being discharged into a large sump. Water from the sump was pumped through an in-line 110W UV steriliser (Tropical Marine Center (TMC), Manchester, UK) before entering the aquaria. Return water was gravity fed into a large free standing filter before being discharged back into the main sump. Fish were monitored daily and any mortalities removed. On 3 March 2014, once all fish had used up their yolk sac and began feeding on exogenous food consistently, equal numbers of offspring from each family were haphazardly assigned into twelve 175 l tanks (keeping parental type

discrete), with six tanks per parental type and 200 fish per tank. Tanks were supplied with water pumped directly from Loch Lomond and held under simulated ambient photoperiod and temperature (9.2 ± 0.2 °C (mean±SD). Tanks were assigned to one of three food treatments (giving 2 replicate tanks per food treatment per offspring ecotype) and fed twice daily on a standard commercial salmon pellet (Biomar, Aarhus, Denmark) for the remainder of the experiment. The three food treatments were high food (approximately 4.3 % body wt. day^{-1}), mid food (approximately 2% body wt. day^{-1}) and low food (0.7% body wt. day^{-1}). High food treatments were based on feed amounts for a given size of fish and temperature recommended by Biomar (Aarhus, Denmark) for maximum growth in trout, with low food and mid food treatments selected to achieve growth rates slightly above maintenance and intermediary between maximal and minimal growth rates respectively, similar to Wysujack et al. (2009).

A.2.3 Methodology For Testing Relative Dominance

The fish were tested for dominance between 25 Sept. 2014 and 15 Nov. 2014, when they were approximately ~7 months old. All trials were conducted in an oval shaped artificial stream channel located at the Scottish Centre for Ecology and the Natural Environment, Scotland (Fig.A.1).

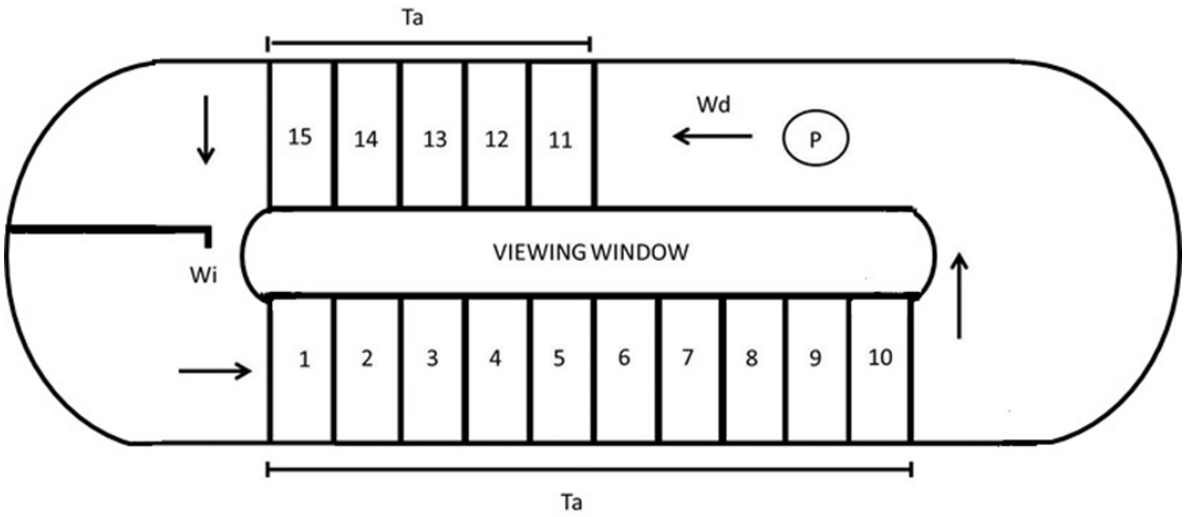

Fig.A.1. Schematic diagram of the experimental stream channel used in this experiment with the straight sections of stream channel divided into 15 equally sized test arenas (Ta); arrows indicate water direction (Wd), with the pump (P) and water inflow (Wi).

Water to the stream channel was pumped at approximately 15 l min^{-1} directly from Loch Lomond, and so was at the same temperature as in the fish's rearing tanks. Straight sections of the stream channel contained a clear glass window which ran the entire length of the channel to allow observations to be carried out. A canopy of dark sheeting between the

straight sections of the stream channel allowed for observations of fish to be carried out from a concealed location and increased the contrast of light to further prevent detection of observer by fish. Both arms were fitted with plastic meshed transverse dividers mounted on wooden frames to give a total of 15 smaller compartments (60cm X 60 cm X 60 cm; Fig.4.1). The substratum was homogenously landscaped with gravel. A 10cm X 5cm rock was placed in the middle of each compartment to produce a single preferred position (so increasing the likelihood of competition between the two fish; Metcalfe et al. 2003). An electric pump ensured relatively uniform velocity (~0.15 ms^{-1}) throughout the artificial stream channel.

All trials used a single pair (one freshwater-resident and one andromous parental type) of fish from the same food treatment in each compartment. Fish were haphazardly selected from the holding tanks, anaesthetized and sized-matched within pairs for fork length (±0.6 mm; Fig. A.2A) and weighed (± 0.13 g; Fig. A.2B).

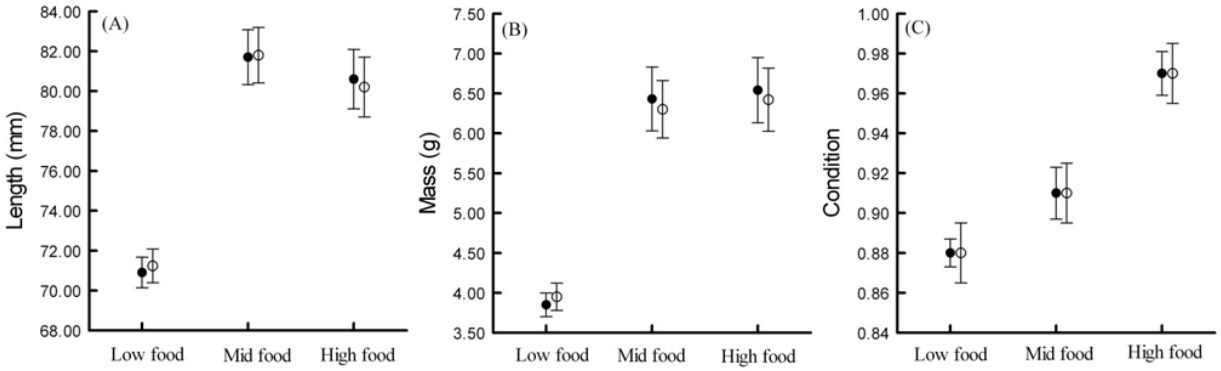

Fig.A.2. The mean (±SE) length (A), mass (B) and relative condition (C) of offspring of freshwater-resident (closed circles) and anadromous (open circles) parents used in the dominance trials. Data shown separately for fish from low, mid and high food treatments. Note that fish were selected to be size-matched within food treatments, but differences between food treatments reflect effects of treatment on growth and condition.

Once sized-matched, one of the two parental types in a pair (type alternated between compartments to prevent any bias due to tagging) was given an alcian blue dyemark on the dorsal fin prior to the experimental trial. The two fish were then released into a compartment of the stream channel and allowed to acclimate for two days prior to a two day period of behavioral observations. Because the artificial stream channel was divided into 15 compartments we were able to run 15 trials simultaneously. All 15 trials in any one run of the experiment were conducted with pairs of fish from the same food treatment. Fish were

fed periodically during the two day acclimation period by flushing a single food pellet through a plastic pipe so that it dropped into the centre of the stream at the upstream end of each compartment.

A.2.4. Data Collection

Four observation sessions were conducted daily (09:00, 11:00, 13:00, 15:00 h) over the course of two days for each pair of fish. Each observation session consisted of first visual recordings of aggression, color, and spatial position for each pair during a 3 min period. After the 3 min period a single food pellet was introduced (as above) into the upstream part of the compartment and a record made of the fish that obtained it. Then a second recording was made of colour and spatial position, followed by a second recording of any aggression over a 3 min period.

Aggression was scored as the total number of aggressive behavioral acts (categorized as fin nips, chases, bites, charges and dorsal displays) exhibited by each individual (Adams et al. 1995) during the 3 min observational periods.

Body colouration is a reliable indicator of social stress in juvenile salmonids, with more subordinate individuals being darker (O'Connor et al. 1999; Kaspersson et al. 2010). Colouration was assessed visually in each fish on a three point integer scale, with brighter fish receiving a score of +1 and darker ones a score of -1. Because colouration was scored twice per trial (before and after feeding) and summed across all 8 observation sessions, a fish's total score for body colouration could range from -16 to +16.

Spatial position was assessed using a protocol developed from earlier research on juvenile salmonids (Metcalfe et al. 2003; Burton et al. 2011). Spatial positions were quantified in three dimensions by marking each axis of a compartment into three equal lengths, using equally distributed marbles on the substrate and marks on the inside viewing window. These marks defined 27 (i.e. 3 x 3 x 3) equal-sized cuboid zones. Since previous research has shown that dominant salmonids occupy central-rear positions, often just above the substrate and behind an object (Metcalfe et al. 2003; Burton et al. 2011), we gave fish that occupied zones in the lower two thirds of the water column at the centre and back of the compartment a score of +1, while those in zones in the corners of the compartment were given a score of -1 and those in all other positions a score of 0 (Burton et al. 2011). Furthermore fish that were observed to be resting against the downstream meshed divider were given an additional score of -1, as were those resting on the substrate. Because spatial position was scored twice per trial (before and after feeding) and summed across all 8 observation sessions, a fish's total score for spatial position could range from -48 (if it was

always resting on the gravel and against the mesh divider in a corner position) to +16 (if it was consistently in a middle position and not touching the mesh or substrate).

The ability to acquire food under competition was scored with reference to the fate of the single food pellets introduced in each observation session. Individuals that made no attempt at acquiring the pellet were given a score of 0, those that attempted but failed to acquire it were given a score of +1 and the fish that succeeded in getting the pellet was given a score of +2. Total scores for competitive ability were summed across all 8 observational periods and ranged from 0 (if an individual made no attempts at feeding) to +16 (if an individual was successful in acquiring the food pellet during every observational period).

Once observations were complete, the fish were anaesthetised, adipose fin clipped (to ensure they weren't used again) and returned to their holding tank and new fish selected; each fish was therefore only used once. A total of 30 pairs from each food treatment were tested during the course of the experiment.

A.2.5. Calculations And Data Analyses

Offspring condition was calculated to ensure there were no differences in condition of individuals in dyadic contests as differences in condition could influence these outcomes. Relative condition factor, K_{rel}, was calculated according to Froese (2006) using the following equation:

$K_{rel} = W/aL^b$

where W is the mass of the individual offspring in grams, L is the fork length in millimetres and a and b are the exponential form of the intercept and slope derived from the regression of mass vs. length plotted on double logarithmic axes for all the offspring combined. These values in addition to the mass and length measurements were used separately as the response variable in a linear model (LM) with parental type and food treatment as explanatory variables.

Individual scores for position, food acquisition, colour and aggression were normalised prior to analysis (by subtracting the mean score of all 180 fish from each individual's score and dividing this value by the standard deviation for all fish; Burton et al. 2011). These normalised scores were then used as the response variable in a linear model (LM) with parental type and food treatment as explanatory variables. Initial mixed models that included pair ID as a random factor showed that pair ID was never significant and explained a negligible percentage of the variation, and so this random factor was dropped from models to make them simpler to interpret. Associations between normalised scores of the four measures (aggression, colour, spatial location and food acquisition) were described using Pearson correlations and then summarised with a principal components analysis (PCA)

summarizing all four behaviors as a general index of fish dominance. Principal Component One (PC1) was found to be highly associated with each of these four separate measurements, with more positive scores indicating more dominant individuals. PC1 scores were therefore analysed using a LM with parental type and food treatment as explanatory variables. All LM models initially included all two way interactions. Interaction terms and independent variables that were not significant at $p>0.05$ were removed from the models, which were re-run to obtain the model of best fit. Initial analyses combined both parental types and all three treatments. If a significant food by parental type interaction was found, data for each food treatment was analysed separately using the above procedure and parental type as a fixed effect. All statistical models were validated by visual inspection of residual plots which did not reveal any obvious deviations from homoscedasticity or normality. Analyses were conducted using the R version 3.0.1 statistical software (R Core Team, 2013).

A.3 Results

A.3.1. Growth And Condition Of Fish

There was a significant effect of prior rearing environment on offspring length and mass ($F_{(2,176)} = 42.60$, $p < 0.001$; $F_{(2,176)} = 38.72$, $p < 0.001$; Fig. 4.2A,B), with pairs drawn from the low food treatments being shorter and lighter compared to those from high (Tukey, $p<0.001$) and mid food treatments (Tukey, $p<0.001$). There was no significant difference in length or mass between mid and high food treatments (Tukey, length: $p=0.55$; mass: $p=0.94$). A significant effect of prior rearing environment on relative condition factor ($F_{(2,176)} = 22.78$, $p < 0.001$) was primarily driven by a lower condition in fish drawn from the low food treatments and mid food treatments compared to those from high (Tukey, $p<0.001$), since there was no significant difference in condition between mid and low food treatments (Tukey, $p=0.19$). The size-matching of fish within each food treatment was successful, since after controlling for food treatment, there were no differences between parental types in length, mass or condition ($F_{(1,176)} = 0.0001$, $p = 0.991$; $F_{(1,176)} = 0.0335$, $p= 0.855$; $F_{(1,176)} = 1.29$, $p = 0.26$; Fig. 4.2: see in methods section pg.49).

A.3.2. Aggression

There was a significant effect of prior rearing environment on aggression score ($F_{(1,174)} = 8.912$, $p < 0.001$; Fig. A.3.A), with pairs drawn from the low food treatments

having a lower aggression score compared to those from high (Tukey, $p<0.001$) or mid food treatments (Tukey, $p<0.01$).

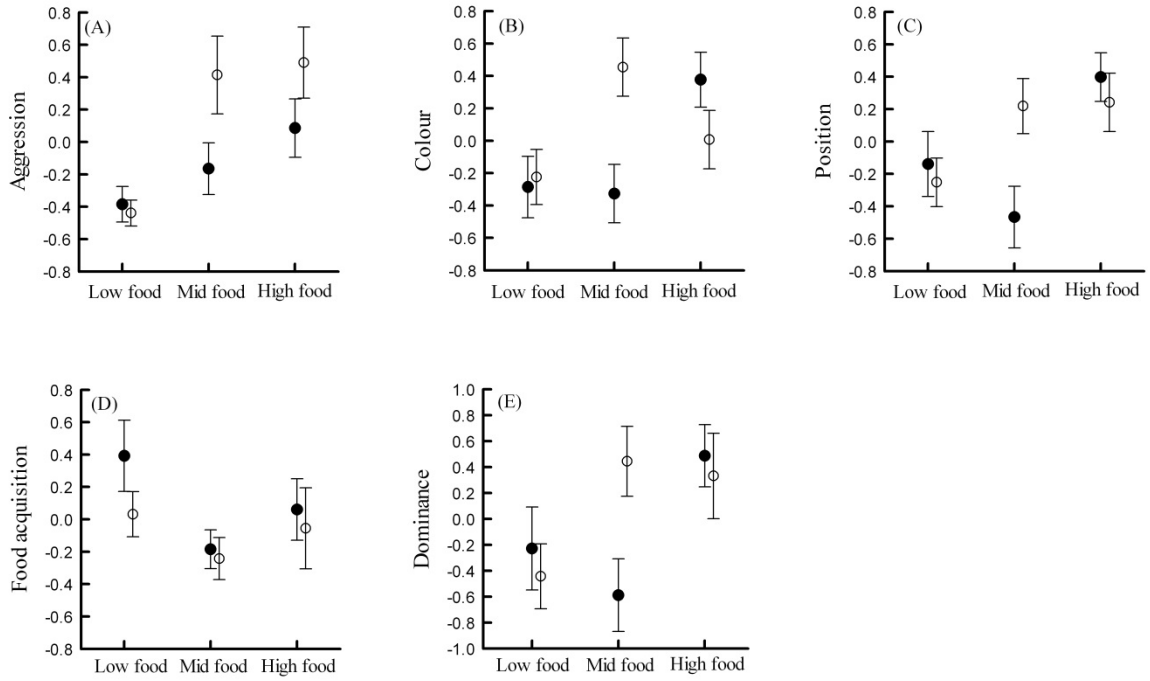

Fig.A.3. The mean (±SE) scores for aggression (A), colour (B), position (C), food acquisition (D) and dominance (E) of offspring of freshwater-resident (closed circles) and anadromous (open circles) parents, plotted separately for pairs from low, mid and high food treatments. Data are plotted as z-scores except for (E) which shows scores for the first principal component in a PCA of the other four variables (A-D). See text for statistical analysis.

There was also an effect of parental type ($F_{(1,174)} = 4.80$, $p = 0.030$), with anadromous offspring having a significantly higher aggression score compared to freshwater-resident offspring. Analysis of the food treatments separately revealed that this overall effect was driven primarily by the significant difference in aggression between the two parental types in mid food pairs ($F_{(1,58)} = 4.16$, $p = 0.046$), as there was no difference in aggression between parental types from the low ($F_{(1,58)} = 0.157$, $p = 0.694$) or high food treatments ($F_{(1,58)} = 2.04$, $p = 0.159$).

A.3.3. COLOUR

There was no significant effect of parental type on offspring colour score ($F_{(1,174)}$ = 1.20, p = 0.27; Fig. A.3.B: see previous page). However, there was a significant effect of prior rearing environment ($F_{(2,174)}$ = 3.43, p = 0.035), with offspring from the low food treatment having the lowest colour score (i.e. darker, more subordinate colouration; Tukey, p=0.03). There was a significant interaction between parental type and prior rearing environment ($F_{(2,174)}$ = 5.46, p < 0.01), indicating that the colour of the freshwater-resident and anadromous offspring differed depending on their prior rearing environment. Analysis of the food treatments separately revealed a significant difference in colouration between the two parental types in pairs of mid food treatment fish ($F_{(1,58)}$ = 9.48, p = 0.003), with freshwater-resident offspring having a more subordinate colouration, whereas there were no differences in colour between parental types in pairs from the high ($F_{(1,58)}$ = 2.34, p = 0.13) or low food treatments ($F_{(1,58)}$ = 0.061, p = 0.81).

A.3.4. POSITION

There was no significant effect of parental type on position score ($F_{(1,174)}$ = 0.931, p = 0.34; Fig. 4.3C: see previous page). However, there was a significant effect of prior rearing environment ($F_{(2,174)}$ = 5.02, p = 0.008), with offspring from low food treatments occupying lower scoring positions compared to offspring from high (Tukey, p=0.01) and mid food (Tukey, p=0.03). There was again a significant interaction between parental type and prior rearing environment ($F_{(2,174)}$ = 3.61, p = 0.029) indicating that the position occupied by freshwater-resident and anadromous offspring differed depending on their prior rearing environment. Analysis of the food treatments separately again revealed a significant difference between the two parental types in the mid food treatment ($F_{(1,58)}$ = 7.04, p = 0.010), with anadromous offspring in that treatment occupying higher scoring positions compared to freshwater-resident offspring; there were no differences in spatial position between parental types in either the high ($F_{(1,58)}$ = 0.443, p = 0.51) or low food pairs ($F_{(1,58)}$ = 0.196, p = 0.66).

A.3.5. FOOD ACQUISITION

There was no significant effect of parental type or prior rearing environment on food acquisition score ($F_{(1,174)}$ = 1.44, p = 0.23; $F_{(2,174)}$ = 2.754, p = 0.07; Fig. A.3.D).

A.3.6. DOMINANCE

Pearson correlations indicated that the four traits recorded in the behavioral observations were significantly correlated (Table A.1).

Behavioural traits				
	Aggression	Colour	Position	PC1
Aggression	-	-	-	0.45
Colour	0.50	-	-	0.55
Position	0.49	0.73	-	0.54
Food acquisition	0.37	0.50	0.49	0.45

Table A.1. Pairwise Pearson's correlation coefficients and PC1 coefficients from PCA analysis, for all four behavioural traits observed. All four traits were significantly correlated with one another (df=178, p<0.001), with the first Principal Component summarizing 64% of the variation.

Principal component analysis indicated that they could successfully be combined into a single PC that summarized 64% of the variation (Table A.1), with high PC1 scores indicating individuals with high aggression, pale coloration, favourable spatial positions and high scores for food acquisition; PC1 was therefore defined as a composite measure of dominance. There was no significant effect of parental type on this dominance score ($F_{(1,174)}$ = 0.904, p = 0.34; Fig. 4.3E: see pg.53). However, there was a significant effect of prior rearing environment ($F_{(2,174)}$ = 3.53, p = 0.031), with offspring reared on low food treatments having a lower dominance score than individuals reared on higher food levels (Tukey, p=0.026). There was also a significant interaction between parental type and prior rearing environment ($F_{(2,174)}$ = 3.06, p= 0.049), indicating that the relative dominance score of freshwater-resident and anadromous offspring depended on their prior rearing environment. Analysis of the food treatments separately revealed a significant difference in dominance between parental types in pairs of mid food treatment fish ($F_{(1,58)}$ = 7.08, p = 0.01), with offspring of anadromous fish having a higher dominance score than those of freshwater-residents, but there were no differences in dominance score between parental types in pairs from either the high ($F_{(1,58)}$ = 2.34, p = 0.13) or low food treatments ($F_{(1,58)}$ = 0.14, p = 0.707).

A.4. Discussion

By rearing offspring from parents with contrasting life histories under different food regimes we have demonstrated that both the effect of migration history of the parents and offspring rearing environment influenced dominance status in competition for feeding territories. Interestingly, we found that anadromous offspring scored consistently higher in position, aggression, colour and overall dominance than offspring of freshwater residents

when fish had previously experienced an intermediate level of food availability, but the two offspring types behaved similarly when reared on low or high food rations. When reared on low food rations both parental types occupied relatively poorer spatial positions and showed very low (= more subordinate) scores for colour, aggression and overall dominance. In contrast, if they had previously experienced high food availability they were observed to occupy more favourable spatial positions, were more aggressive and had more dominant colouration.

The positive relationship observed between correlates of dominance and rearing environment suggests that the foraging and territorial strategy adopted is dependent on the individual's previous experience of the profitability of the environment. It has previously been argued that the strength of territorial defense should depend on resource availability, with greatest resource defense at intermediate levels (Myers et al. 1979; Toobaie & Grant 2013). Individuals from the low food treatment, which were in poorer average body condition, may have been less able to engage in costly aggressive interactions (Johnsson et al. 1996). As a result they were likely adopting an "energy minimising" or "sit and wait" strategy, whereby individuals sacrificed the option of obtaining a territory in a good feeding position to avoid the energetic costs of both swimming against the water flow and potential battles over territories (as seen by the low aggression scores in this treatment group). Since investment in territorial aggression can be costly for growth (Vøllestad & Quinn 2003), minimisation of costs may have been the best strategy for these fish (Metcalfe 1986). While these fish showed less inclination to establish a feeding territory, they nonetheless tended to acquire more food than pairs from the higher food treatments, suggesting that short-term gain was more important than the long-term benefits of a territory. In contrast, individuals from the high food treatment, which were in better body condition, tended to adopt a more active and aggressive strategy (i.e. competing for the best foraging position in the centre of the water column) that may be geared toward longer-term access to food.

The differences in foraging and competitive strategies based on an individual's prior experience of access to food that were observed in our study have parallels in the study by Sundström et al. (2003), which showed that brown trout reared in a hatchery were more aggressive in territorial defense than wild-reared conspecifics. These findings may help to explain inconsistences in the relationship between dominance status and growth found in other experimental studies. For example, juvenile Atlantic salmon with higher social status have been shown to have higher grow rates than subordinates when fed *ad libitum* from a point source in the laboratory (Metcalfe et al. 1989) but have similar or lower growth rates

relative to subordinates when food predictability decreases (Huntingford & Leaniz 1997; Höjesjö et al. 2002; Reid et al. 2012).

Although traits related to dominance did not differ between offspring from freshwater-resident and anadromous parents if they had been reared on high or low food rations, there was a significant difference in dominance if they had been reared at an intermediate food level (with offspring from anadromous parents behaving in a more dominant and competitive manner). This asymmetry suggests that the two parental types differ in the environmental threshold at which they adopt a more competitive and aggressive foraging strategy, with offspring from anadromous parents switching to territorial behaviour at a lower food level. One possibility for the observed difference between parental types is inherent differences in foraging motivation driven by asymmetries in physiology or projected life history. Differences in motivational state linked to hunger have been shown to increase aggression and strengthen social interactions in birds (Andersson & Ahlund 1991) and fishes (Dill et al. 1981; Johnsson et al. 1996), and it may be that the offspring of anadromous parents have either a different food requirement or threshold for territoriality, leading to the observed differences in aggressiveness between parental types on mid food rations.

Another possibility for the parental type by food level interactions could be the timing of the experiment with respect to whether or not individuals were destined to migrate. Given their size at the time of the experiment, the earliest that any of the experimental fish would migrate to sea would be in the following spring. Morinville & Rasmussen (2003) demonstrated that individual migrant brook trout had higher food consumption rates and lower growth efficiencies in the year leading up to migration compared to sympatric resident brook trout, suggesting that the migrant brook trout had higher metabolic costs. Furthermore, Thorpe et al. (1998) proposed that the decision to migrate in the closely-related Atlantic salmon occurs soon after mid-summer (around the time of this study): fish that fall below their individual threshold for growth rate at this time subsequently decline in appetite, cease growth and defer migration, while those individuals who are above the growth threshold maintain their appetite throughout late summer and autumn and undergo migration the following spring. If this pattern of migration holds for brown trout, then all fish in the low food treatment may have already failed to reach the threshold triggering migration. However, if the tendency to migrate is partially genetically determined (Berthold 1988; Berthold & Pulido 1994), then under the mid food treatment offspring of migrants might have had a lower growth threshold triggering migration, so would have a greater motivation to acquire feeding territories so as to be able to maintain their growth rate. Meanwhile all fish reared

on the high food treatment may have passed their respective thresholds, leading to aggressive and dominant behavior being expressed by both parental types in preparation for migration the following spring.

One potential caveat to our study is that we were unable to determine whether the differences between offspring behavior were primarily due to genetic or maternal effects, but this would be difficult to establish given that the resident-anadromous dichotomy by its very nature prevents the use of the standard approach of rearing the parents in a common garden to rule out maternal effects.

In conclusion, the results of this study demonstrate that both the life history strategy of the parents and the rearing environment of the offspring may have a significant effect on a range of dominance related traits. Therefore, we suggest that inherent differences in dominance-related traits, when coupled with spatial variation in environmental productivity, may play a significant role in the perpetuation of non-breeding partial migration within populations.

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